

BIOLOGICAL APPLICATIONS AND OPPORTUNITIES FOR NANOSCIENCE

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National Center for X-ray Tomography

University of California, San Francisco and
Lawrence Berkeley National Laboratory



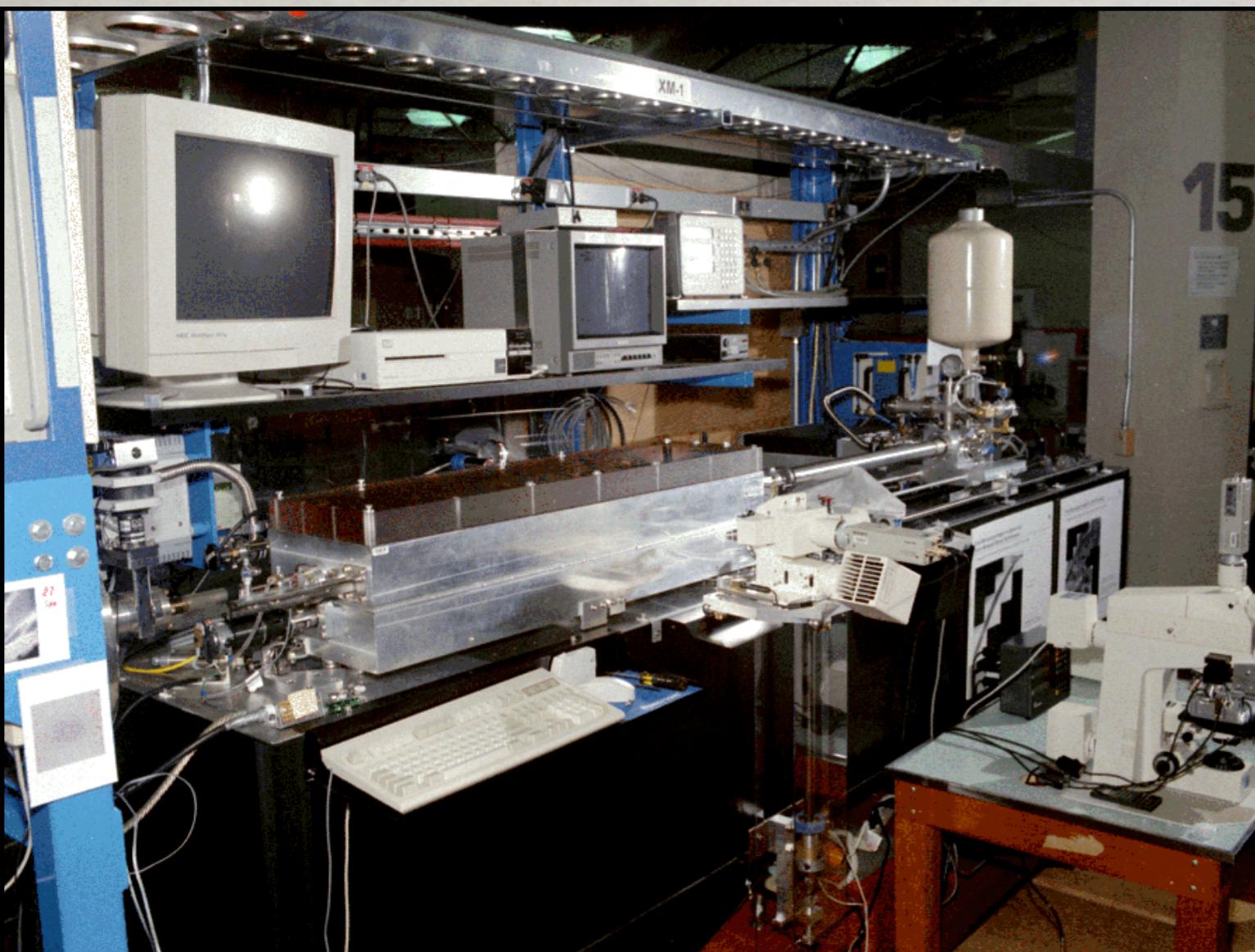
OUTLINE

- X-ray Tomography: 3-D images of whole cells in their natural environment at better than 50 nm resolution
- Enabled by modern synchrotrons and nanofabrication of zone plate optics
- Continued developments needed to achieve better resolution, greater efficiency
- Quantum Dots: Tumor cell migration and invasion

Lawrence Berkeley National Laboratory

Advanced Light Source





Soft X-ray Microscope (XM-1) at the ALS Lawrence Berkeley Lab

XM-1



2.4 nm λ
517 eV

Center for X-ray Optics
David Attwood
Erik Anderson

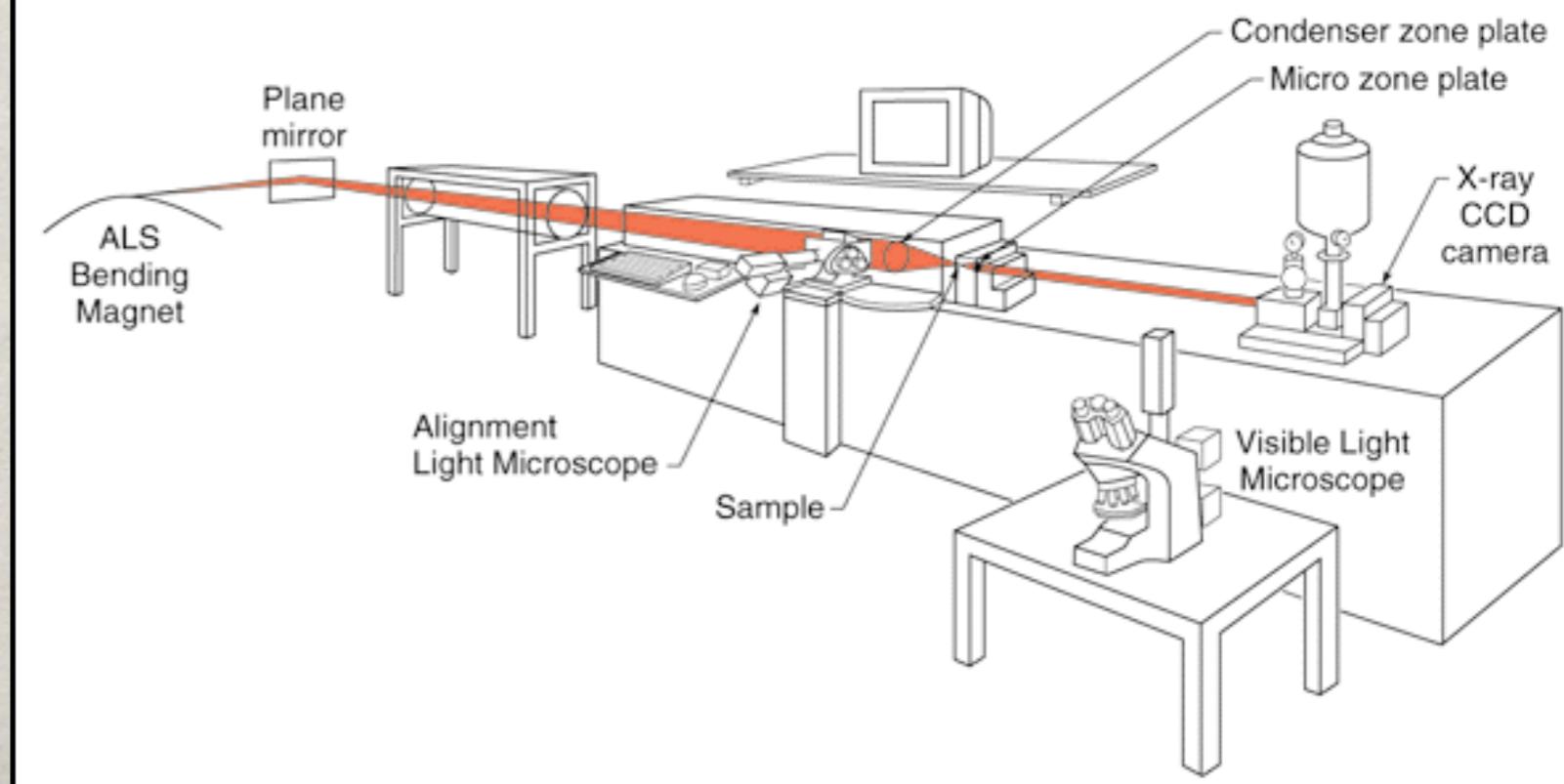
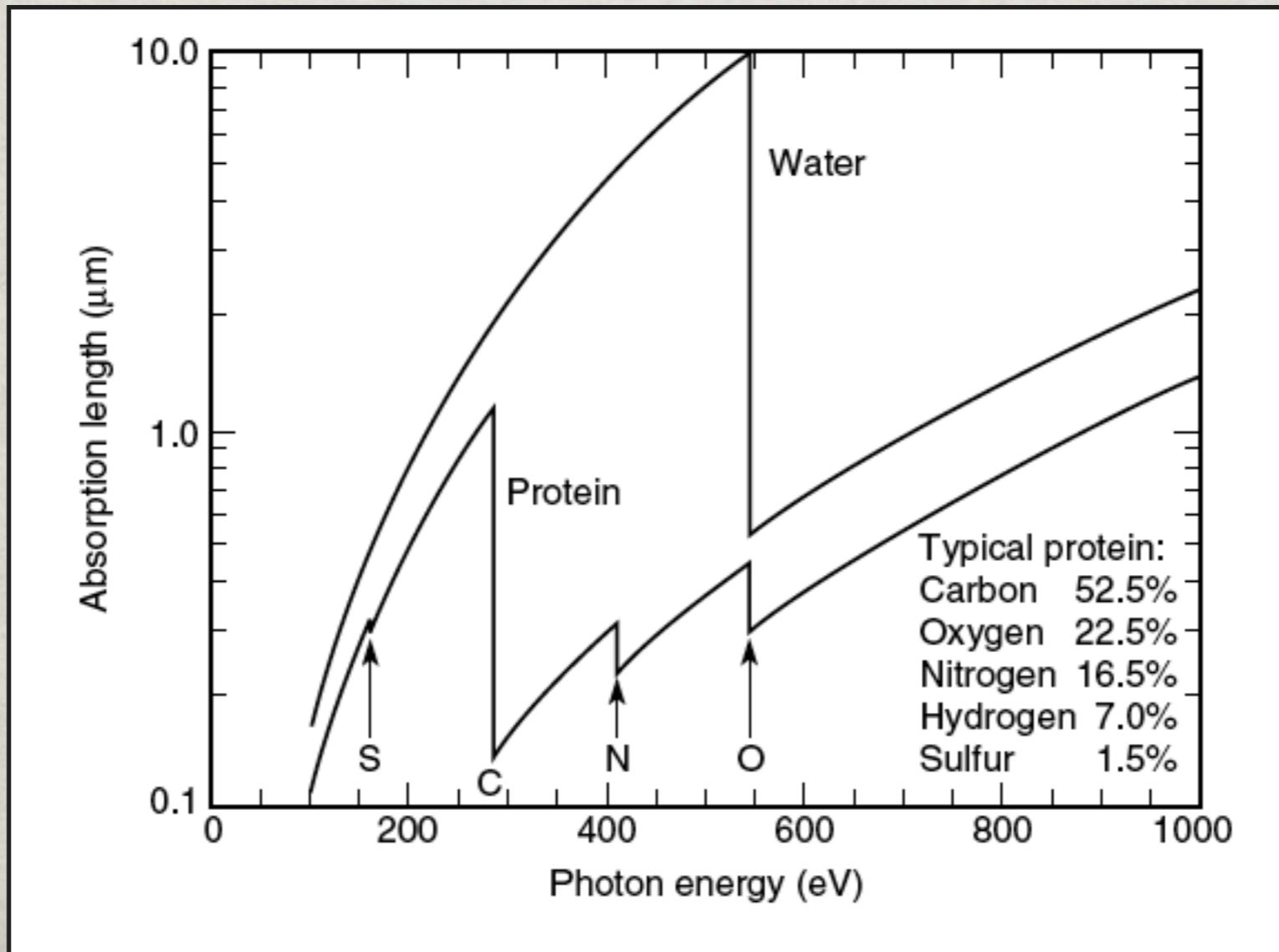
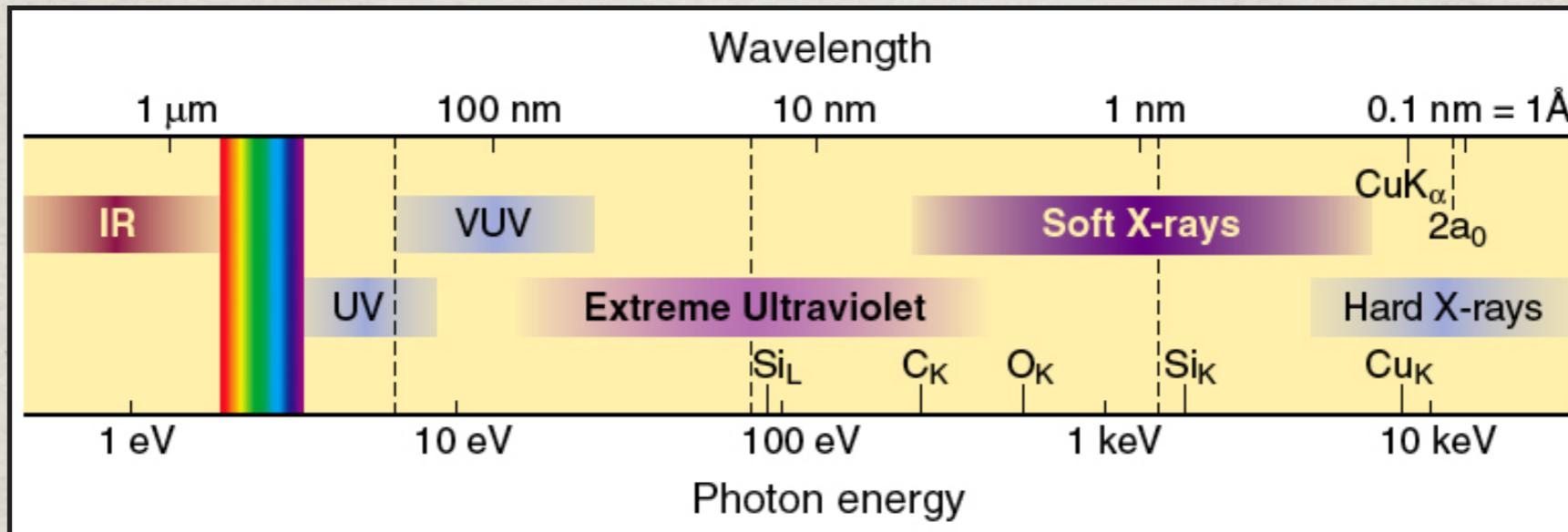
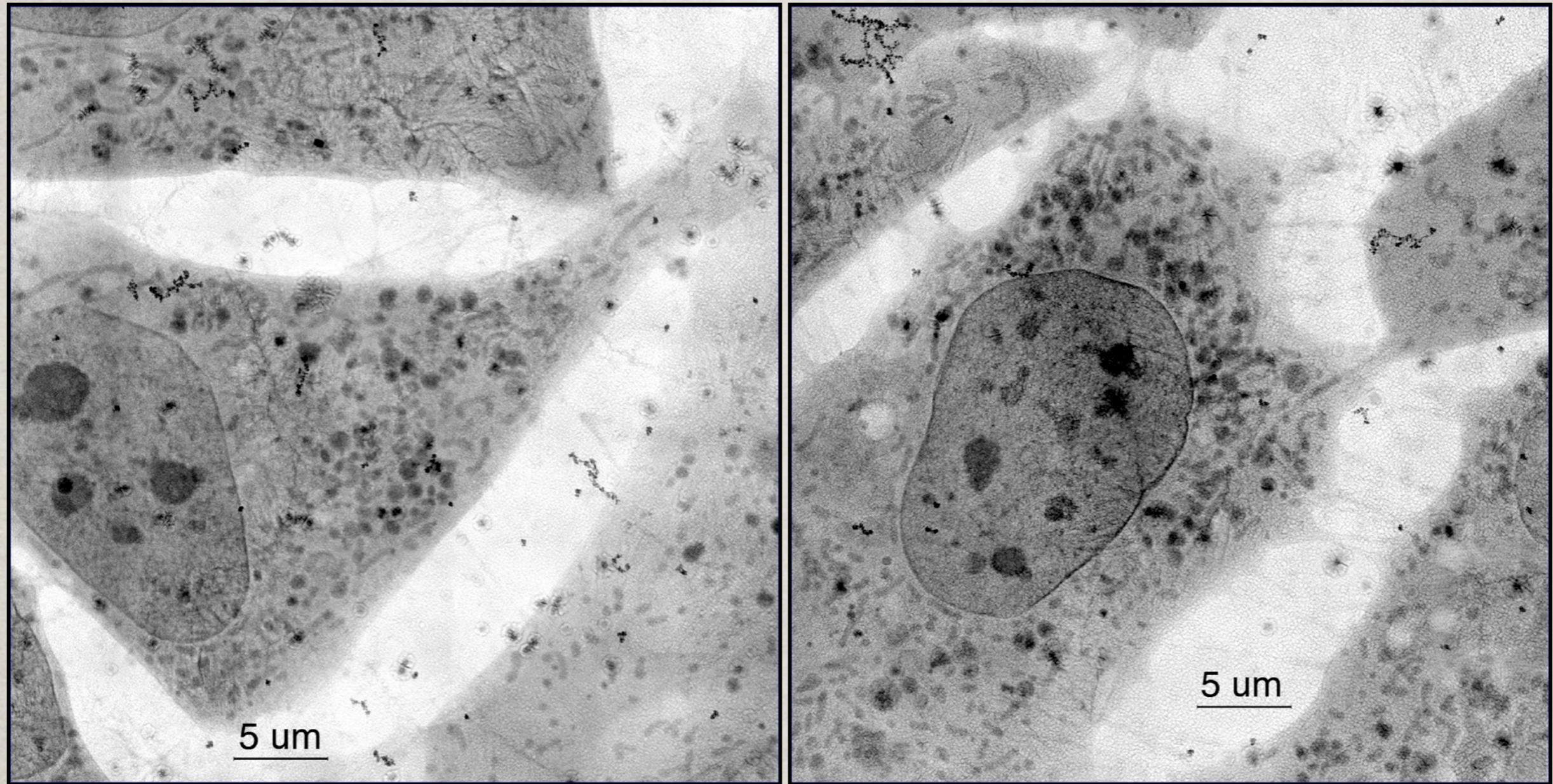


Image in Water Window ~ Natural contrast

Between K shell absorption edges of oxygen (543 eV; 2.3 nm) and carbon (284 eV; 4.4 nm)



Cryo X-ray Microscopy of NIH 3T3 Fibroblasts



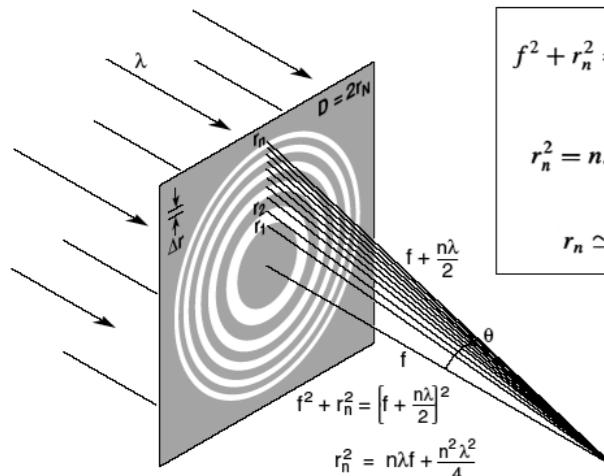
Whole cells - no fixatives, stains, or contrast enhancement reagents

Natural Contrast !!

Meyer-Ilse, W., Hamamoto, D., Nair, A., Lelievre, S.A., Denbeaux, G., Johnson, L., Pearson, A.L., Yager, D., LeGros, M.A., and Larabell, C.A. (2001). *J. Microscopy*. 201, 395-403.



A Fresnel Zone Plate Lens



$$f^2 + r_n^2 = \left(f + \frac{n\lambda}{2}\right)^2 \quad (9.8)$$

$$r_n^2 = n\lambda f + \frac{n^2\lambda^2}{4} \quad (9.9)$$

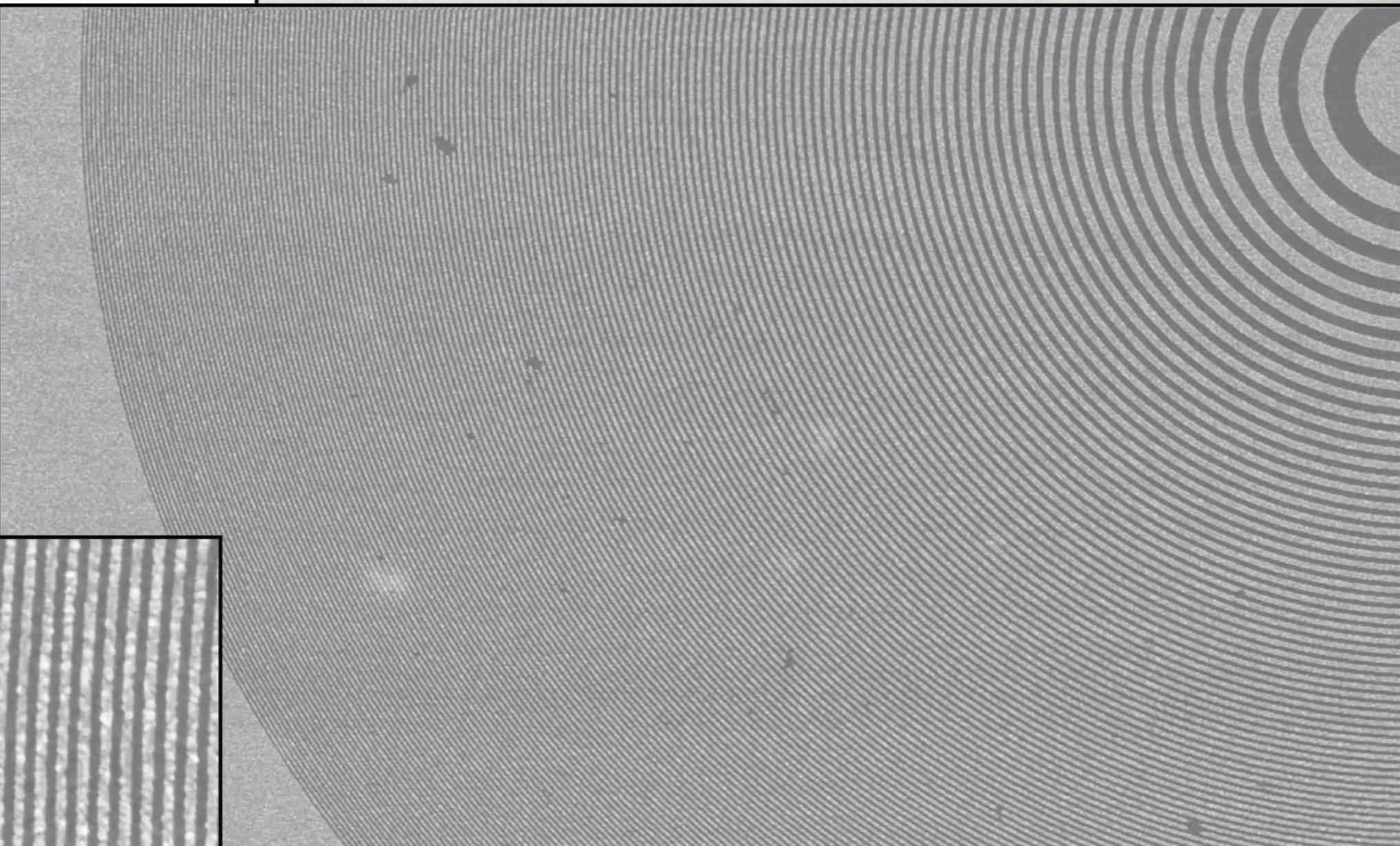
$$r_n \simeq \sqrt{n\lambda f} \quad (9.10)$$

Professor David Attwood
AST 210/EECS 213
Univ. California, Berkeley

$D_r \approx 25 \text{ nm}$
 $D = 63 \mu\text{m}$
 $N = 618 \text{ zones}$
 $f = 650 \mu\text{m}$
 $\text{NA} \approx 0.05$
 $\text{@ } 2.4 \text{ nm } \lambda$

Zone Plate Lenses

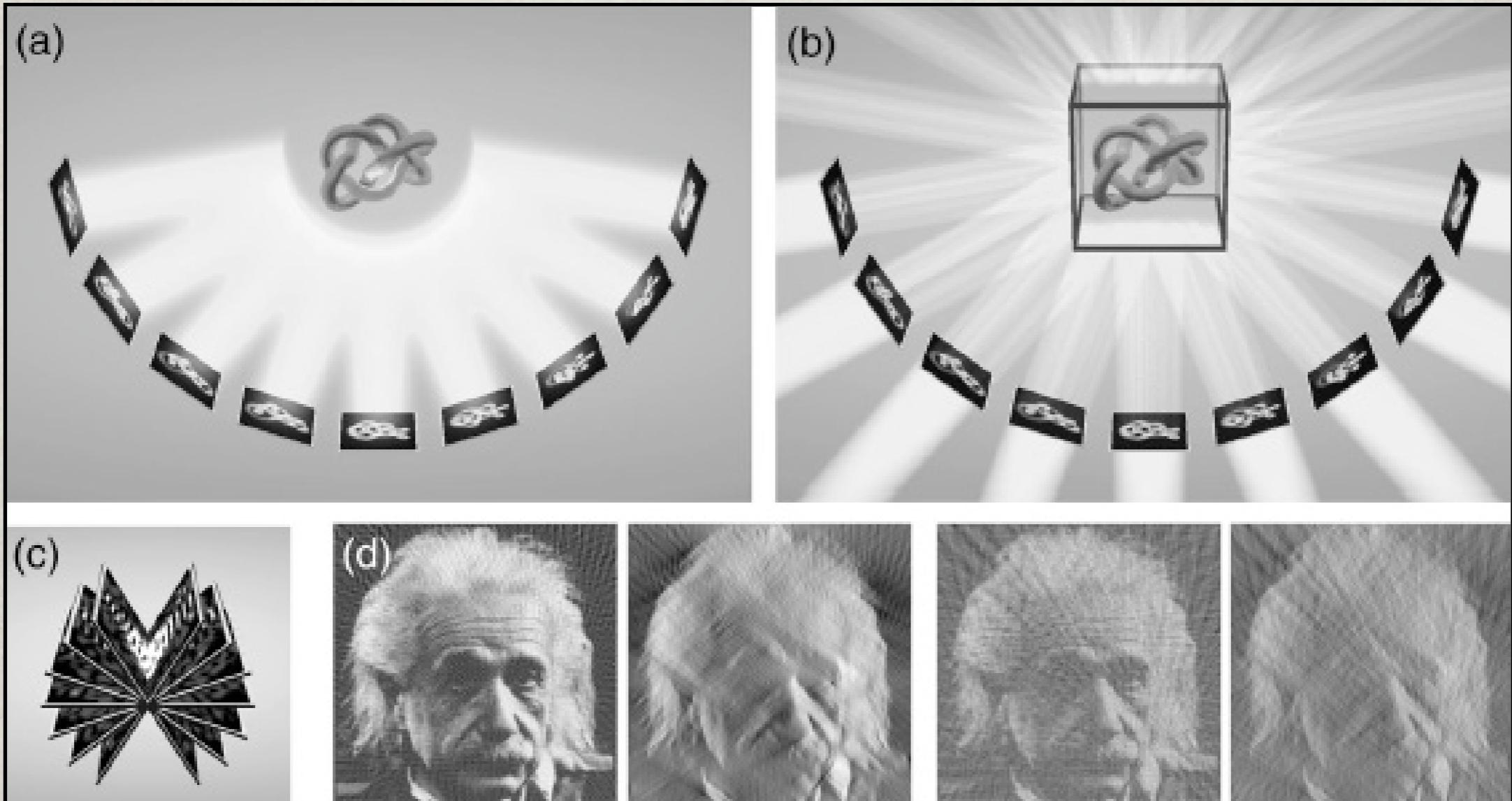
15 nm Resolution (Chao et al., Nature, in press)



E. Anderson, D. Olynick, B. Harteneck, A. Liddle,
W. Chao, D. Attwood; CXRO

Tomography

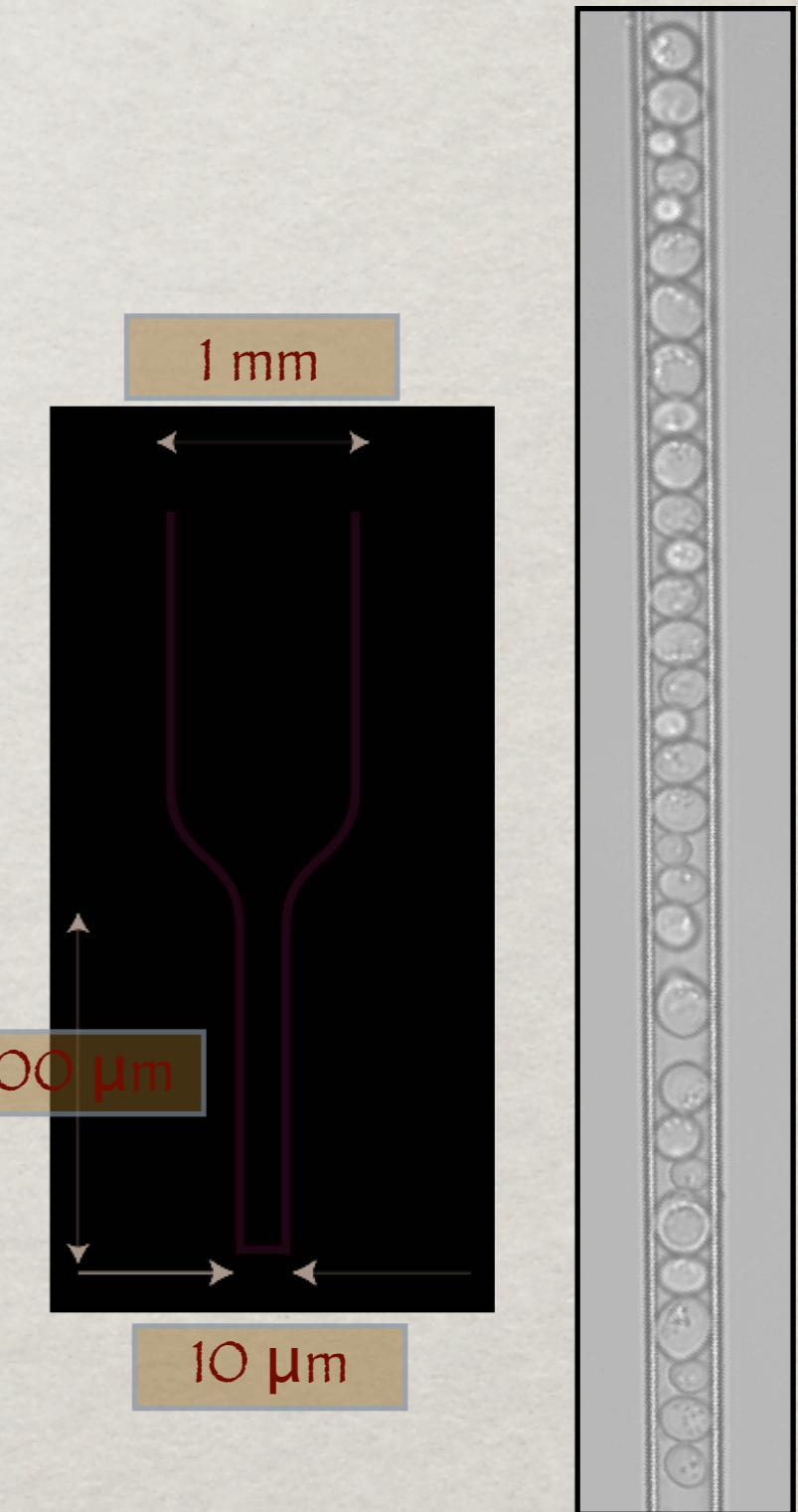
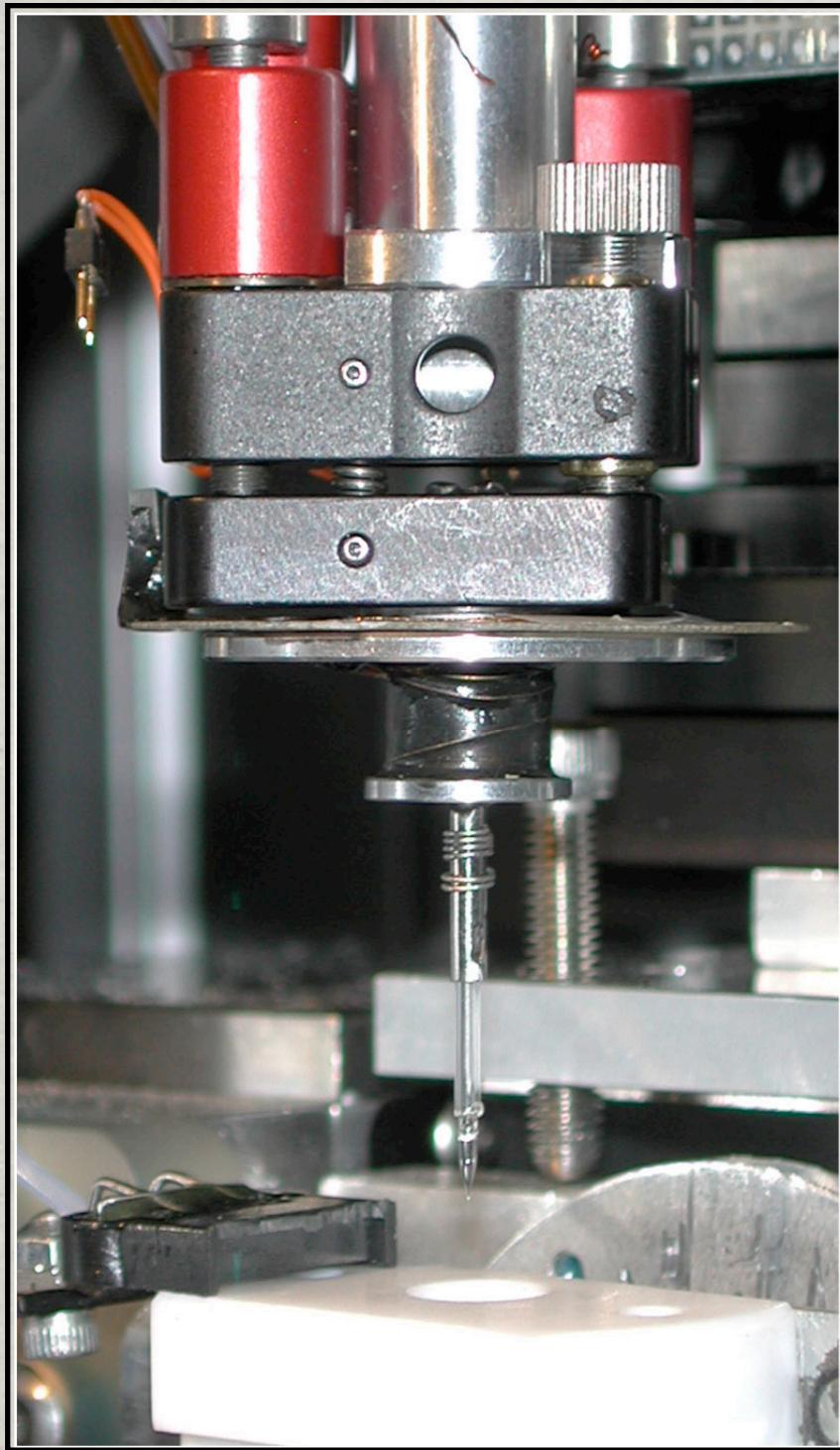
Need multiple images from maximum number of angles



$\pm 90^\circ, 2^\circ$ rot $\pm 60^\circ, 2^\circ$ rot $\pm 90^\circ, 5^\circ$ rot $\pm 60^\circ, 5^\circ$ rot

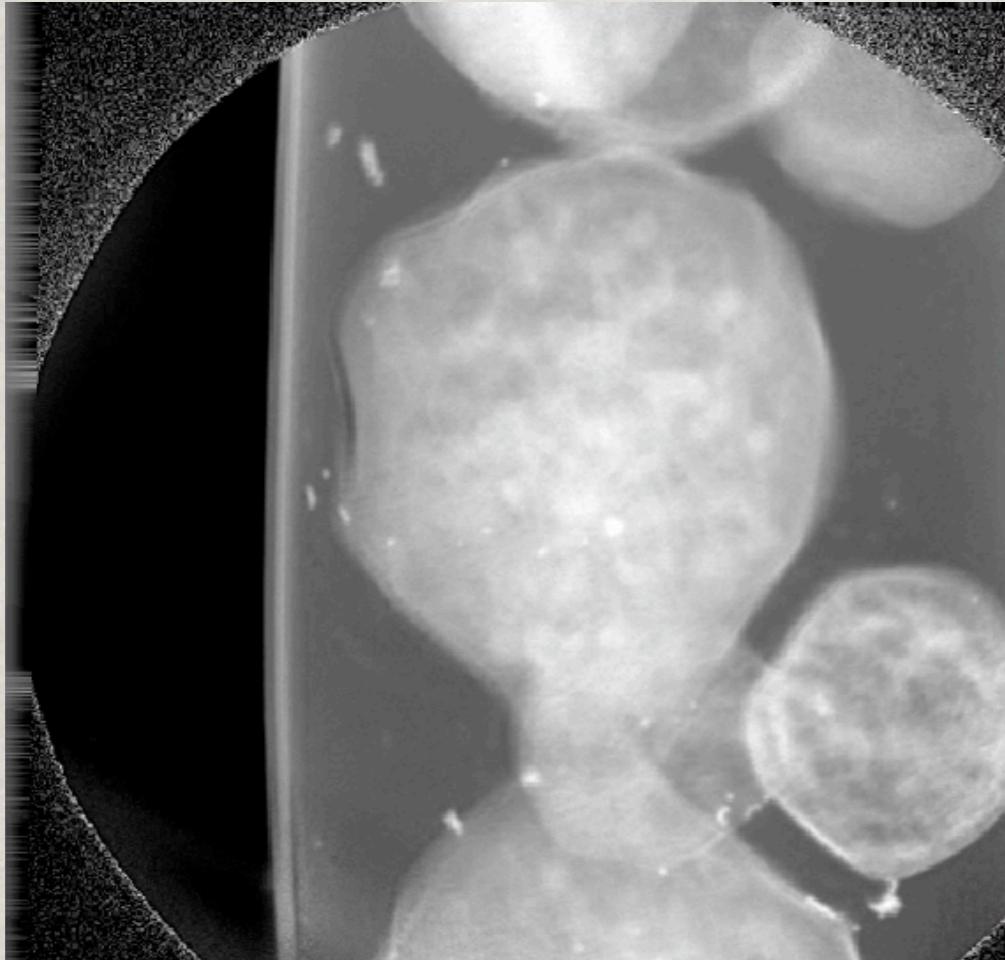
Cryo X-ray Tomography

Cells in capillary

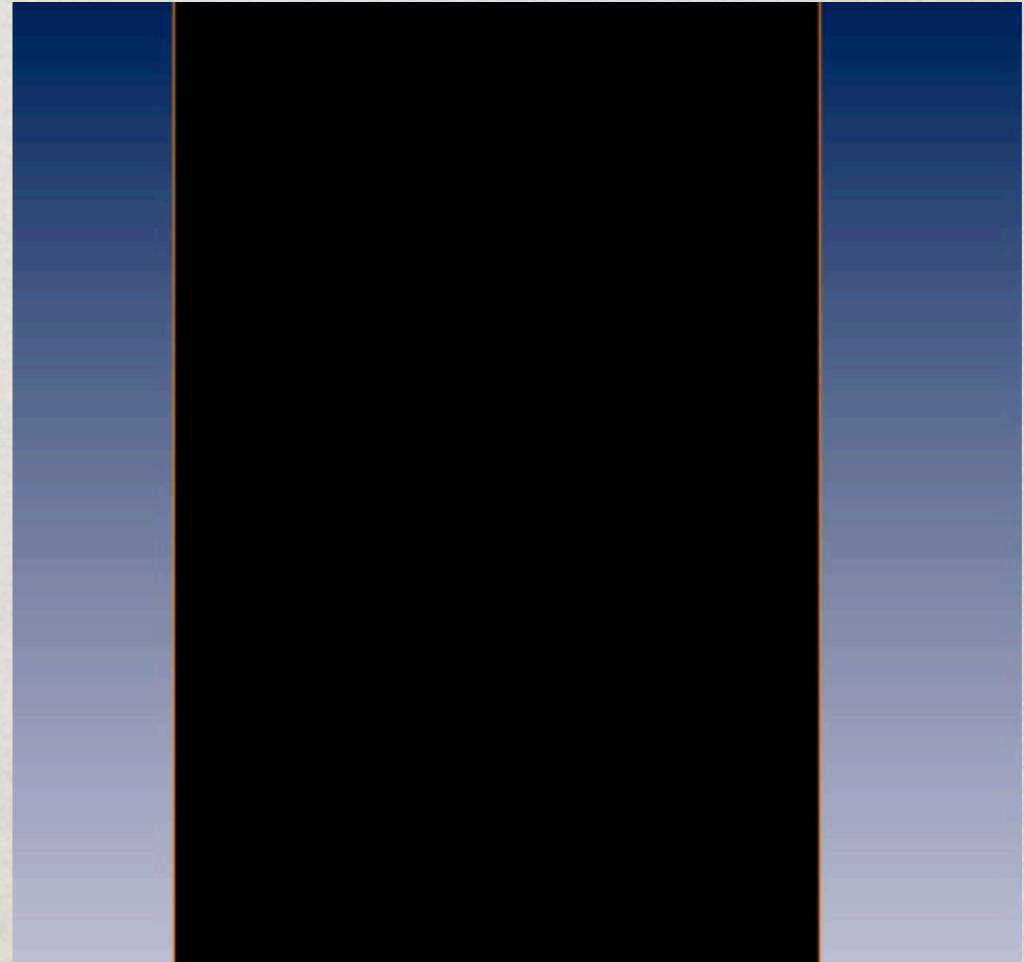


Saccharomyces cerevisiae

45 images collected at 4-degree intervals



Projection images
(60 nm gold balls as fiducial markers)

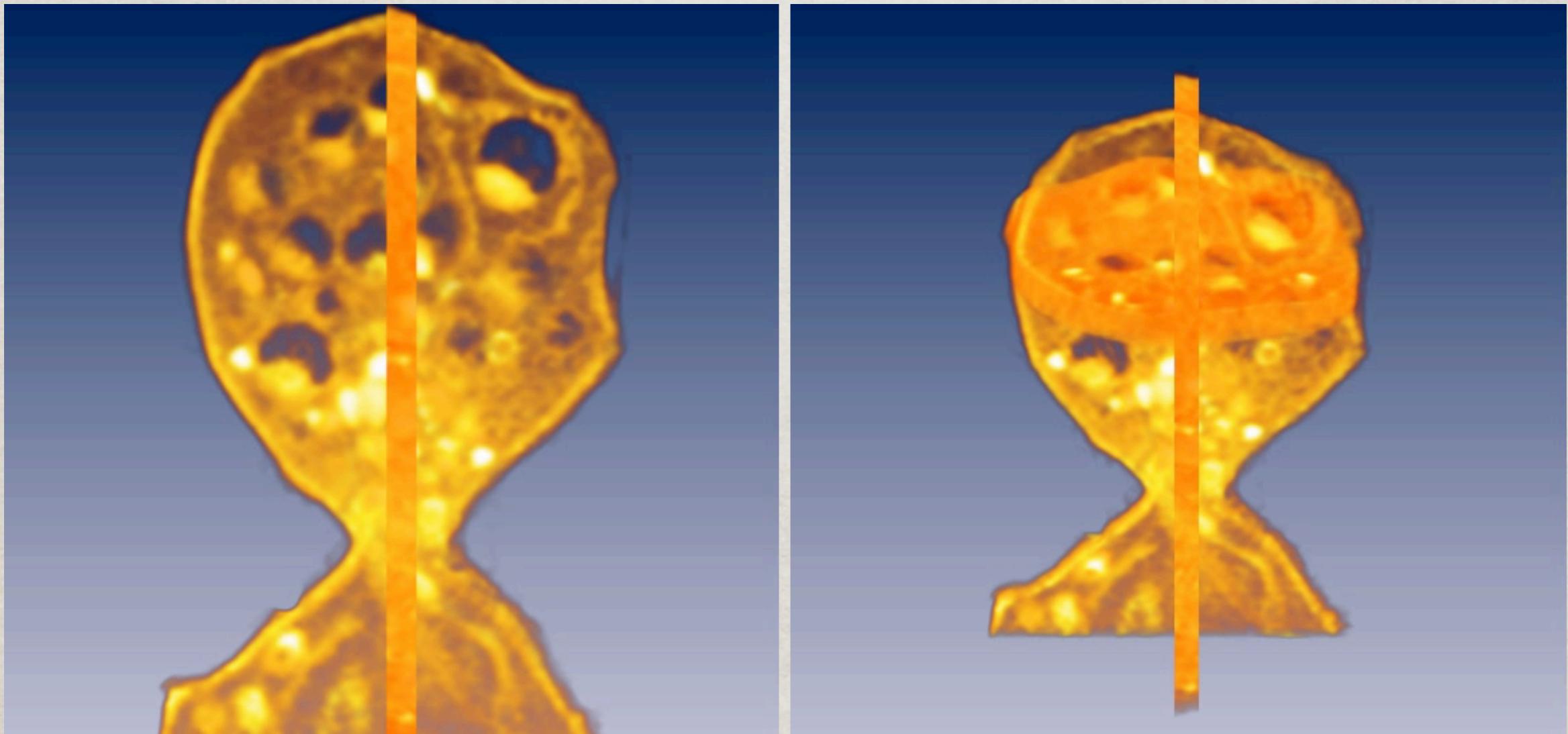


Sections through the
reconstructed data

Yeast bud = 3 microns diameter

Saccharomyces cerevisiae

45 images collected at 4-degree intervals

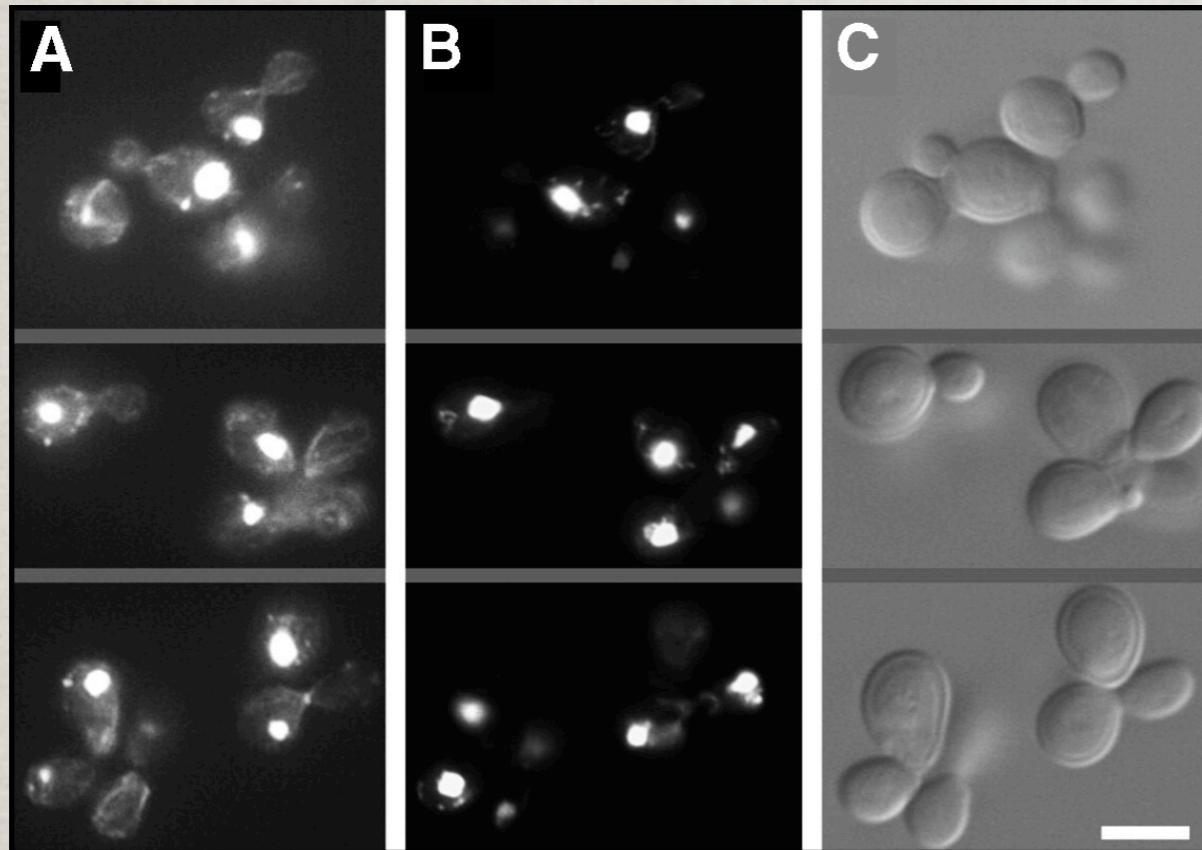


2 sections from reconstructed data

Yeast bud = 3 microns diameter

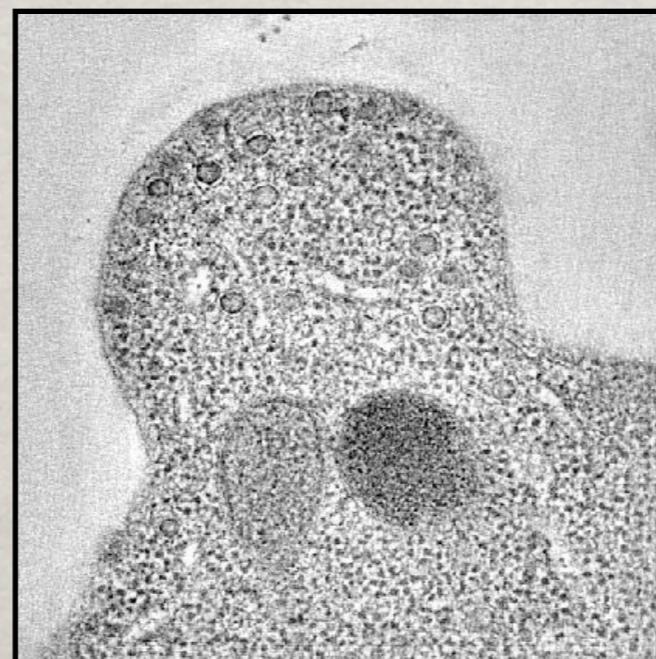
Saccharomyces cerevisiae

Light Microscopy

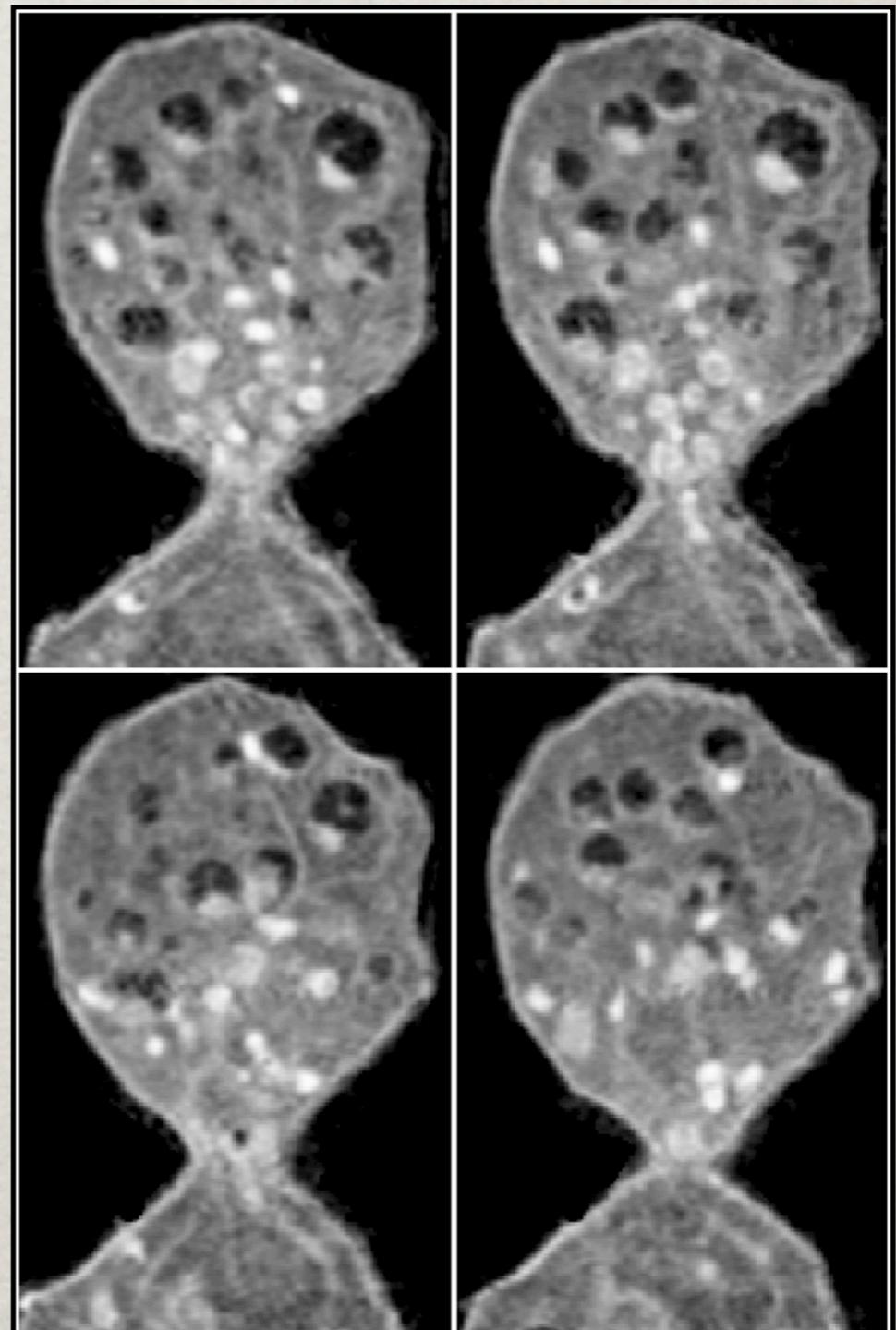


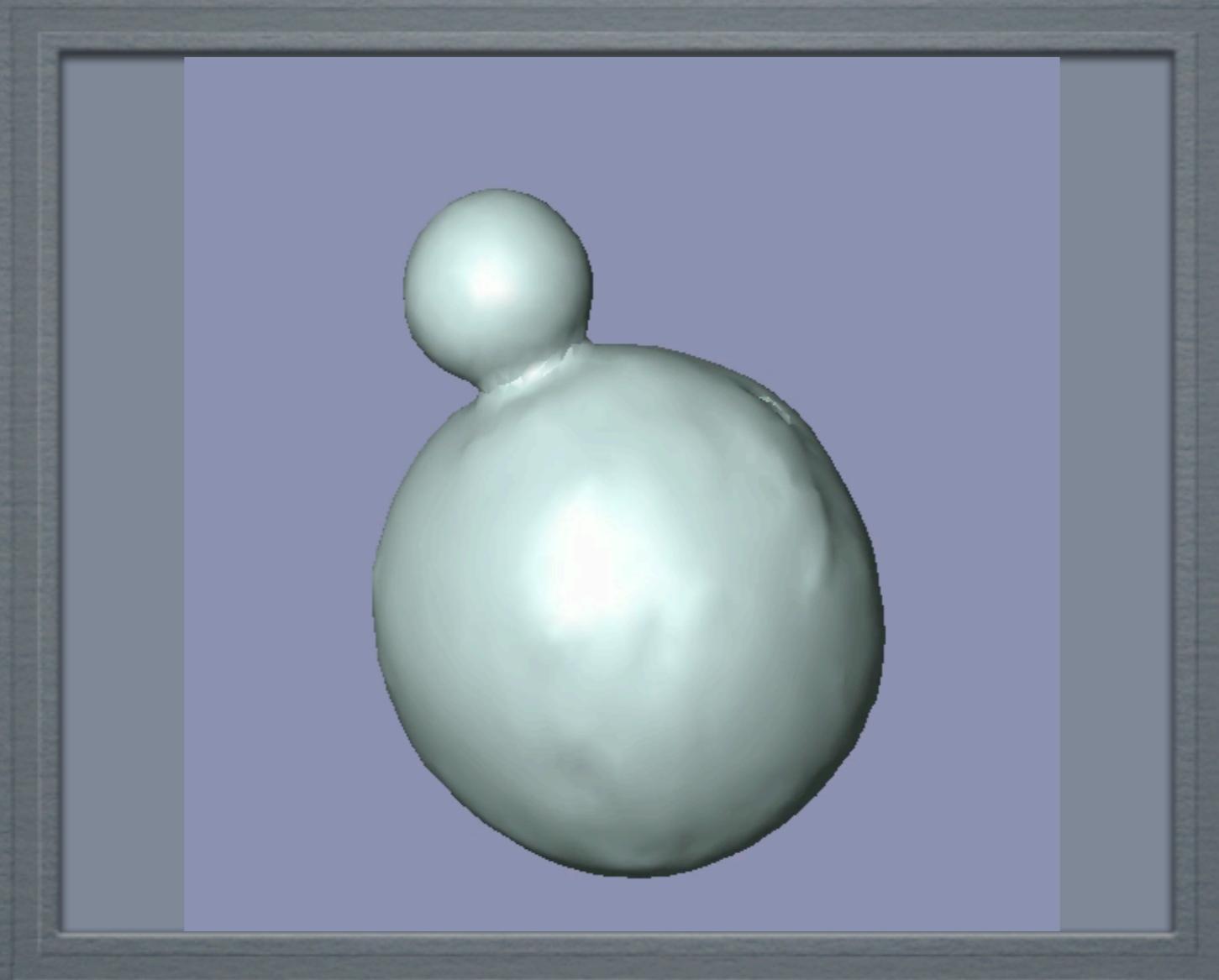
Electron Tomography

250 nm thick section
of yeast cell



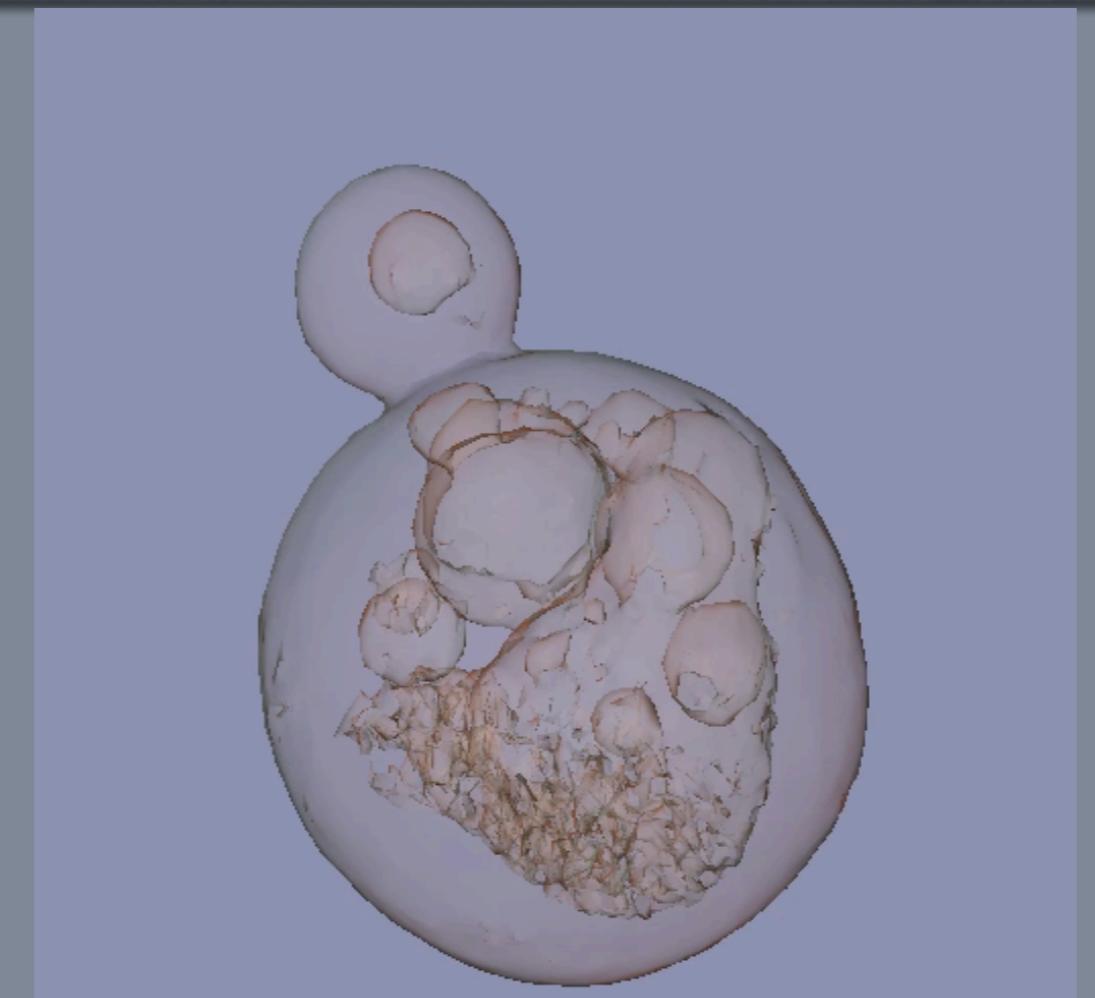
X-ray Tomography





Opaque
surface
extraction

Saccharomyces cerevisiae



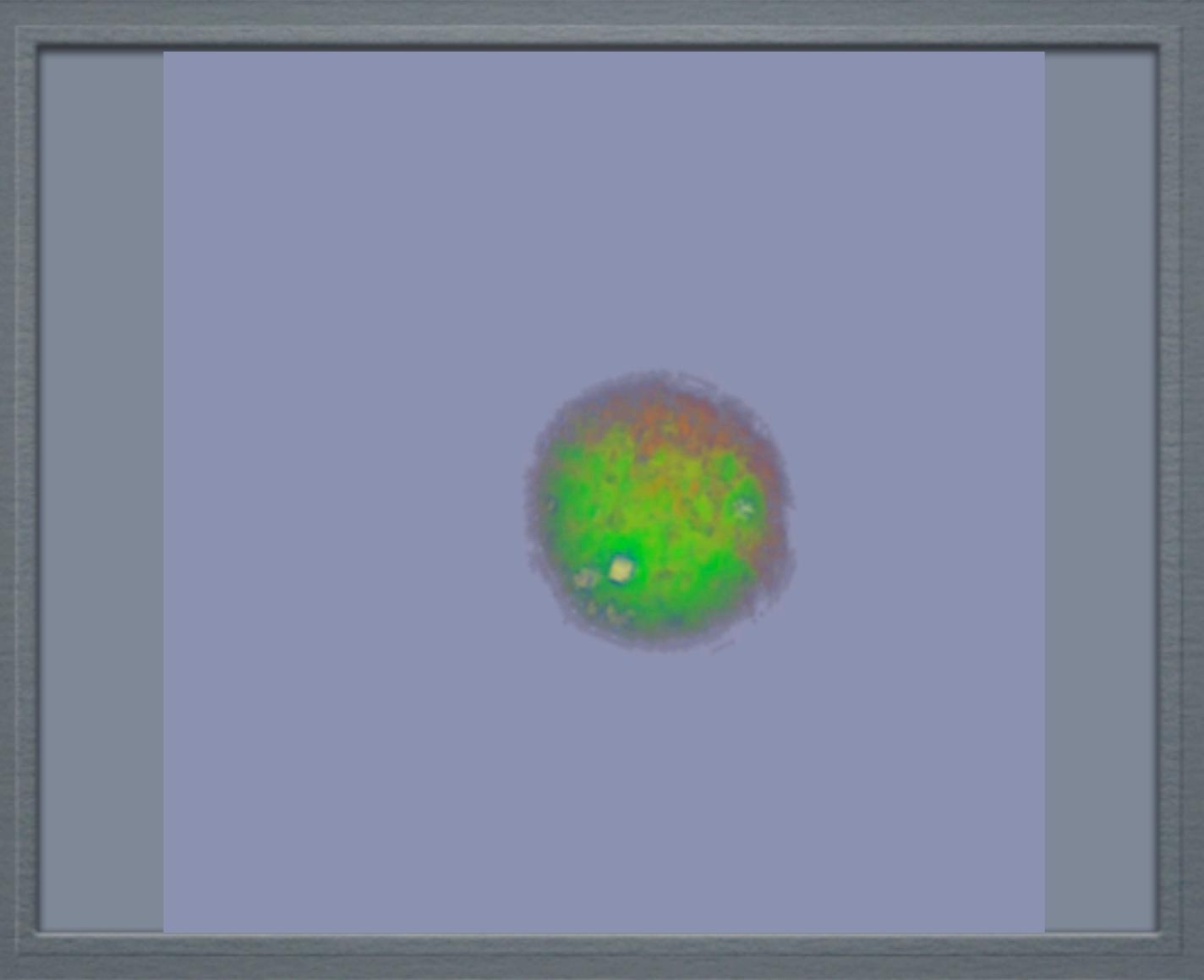
Transparent
surface
reveals
internal
vesicles

Saccharomyces cerevisiae

C.A. Larabell & M. A. Le Gros (2004). Molecular Biology of the Cell, 15(3), 956-962

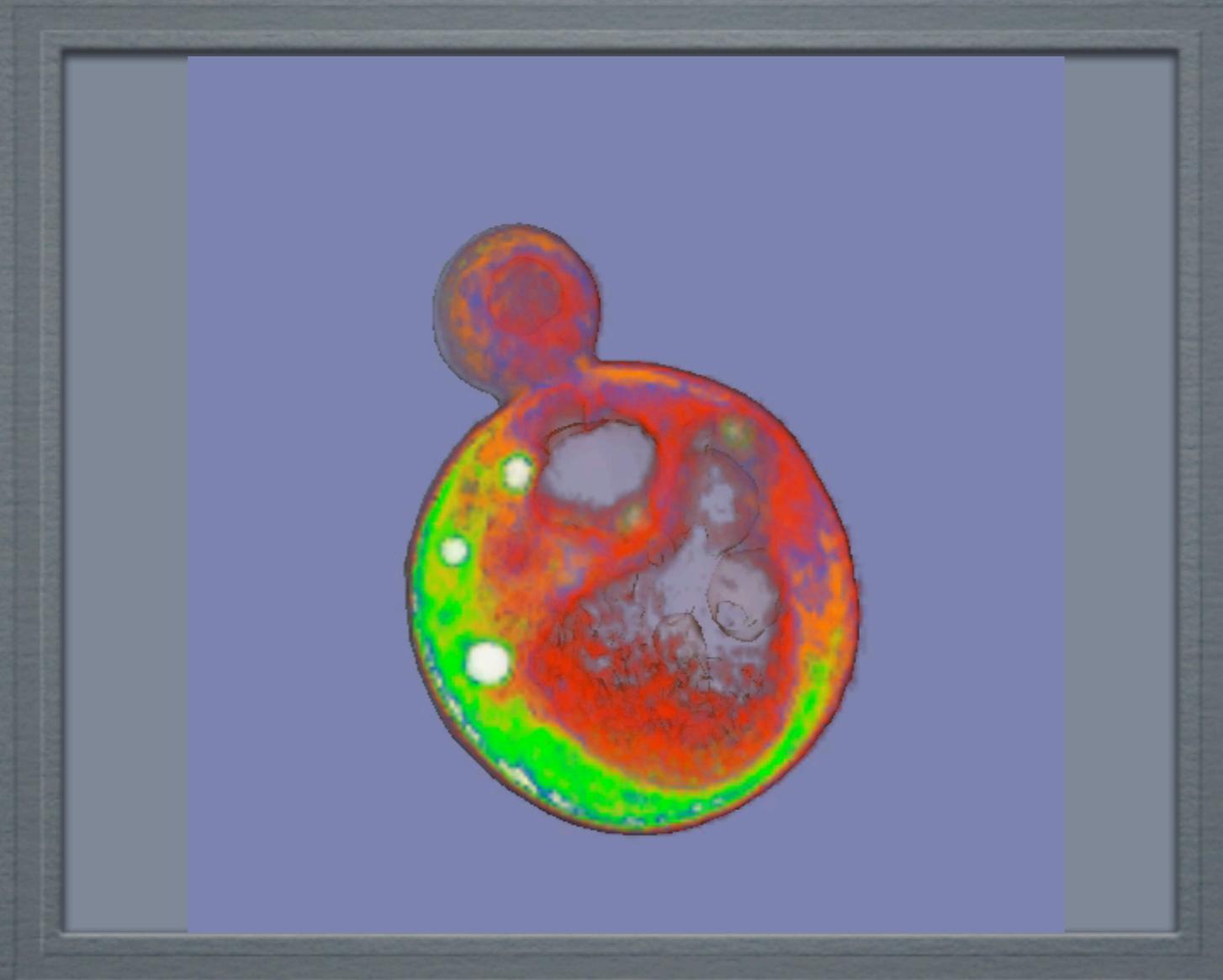
Volume rendered

Color-coded using
x-ray absorption
coefficient



white = dense lipid
droplets
gray = less dense
vacuoles
green, orange, red =
structures of
varying densities

Saccharomyces cerevisiae

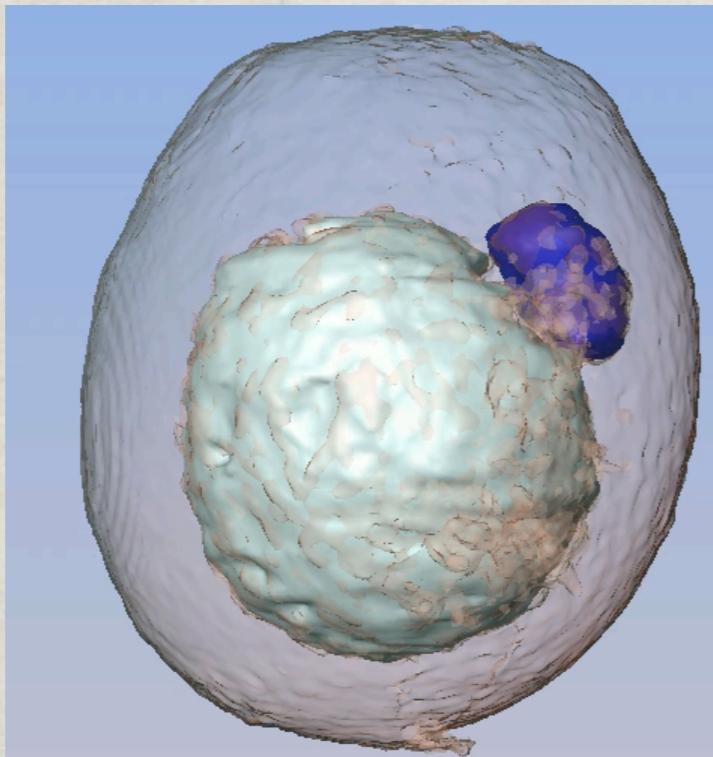


Volume rendered
Color-coded
using x-ray
absorption
coefficient
superimposed on
views of internal
vesicles

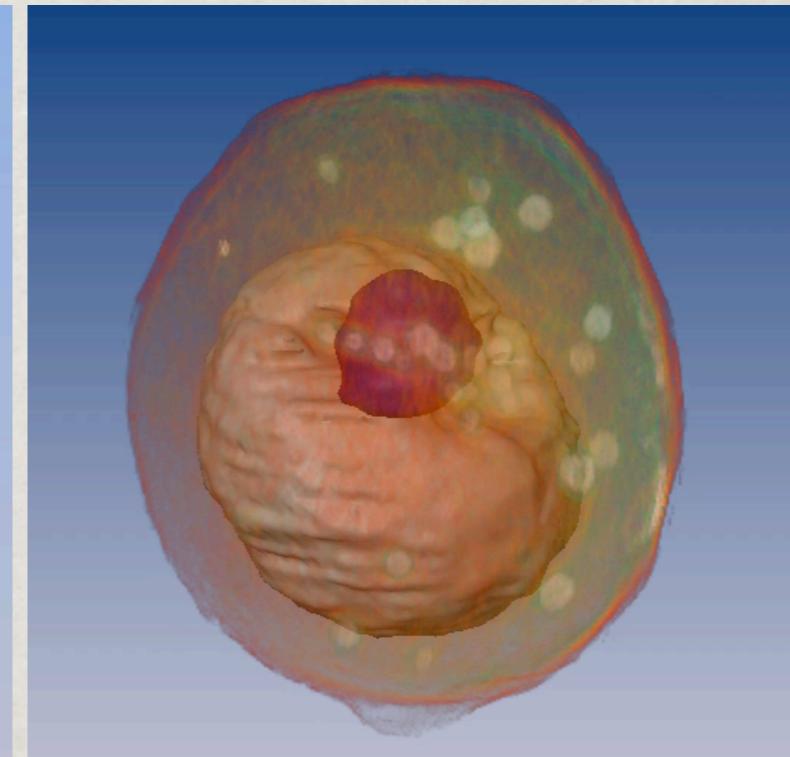
Saccharomyces cerevisiae

Saccharomyces cerevisiae

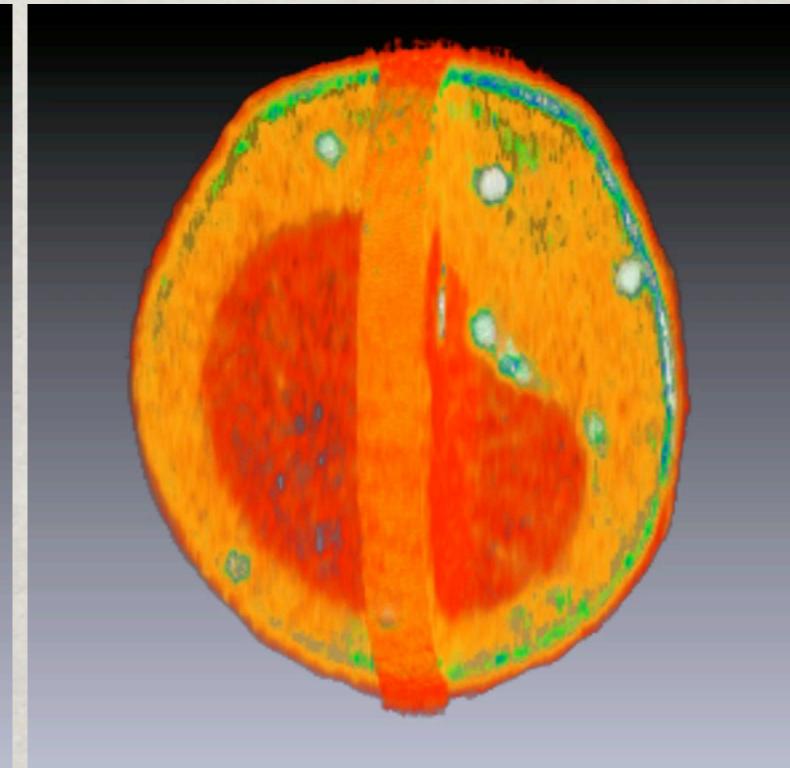
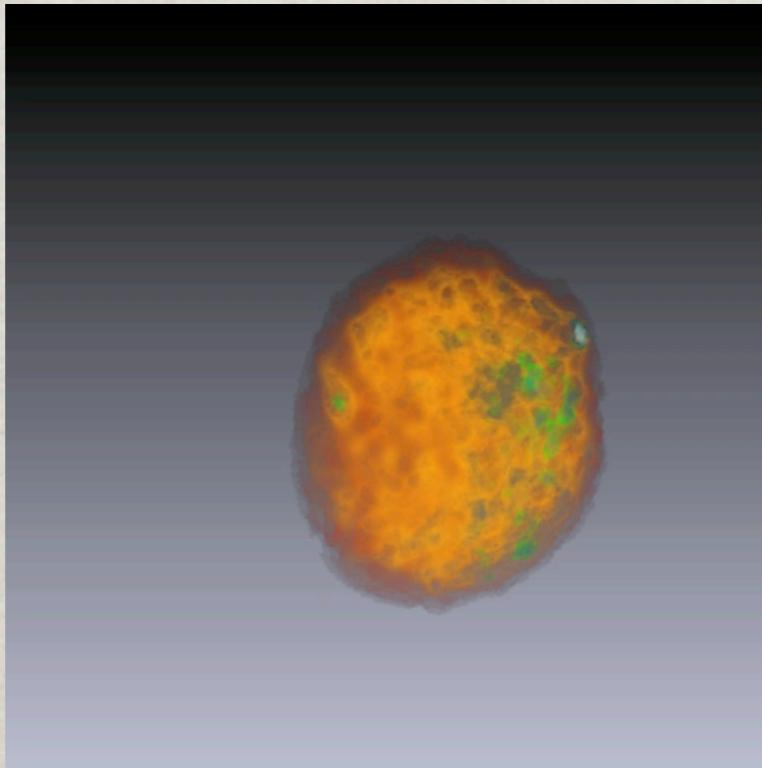
Translucent outer surface plus opaque surfaces showing internal organelles



Volume rendered combined with translucent outer surface & opaque surfaces showing internal organelles

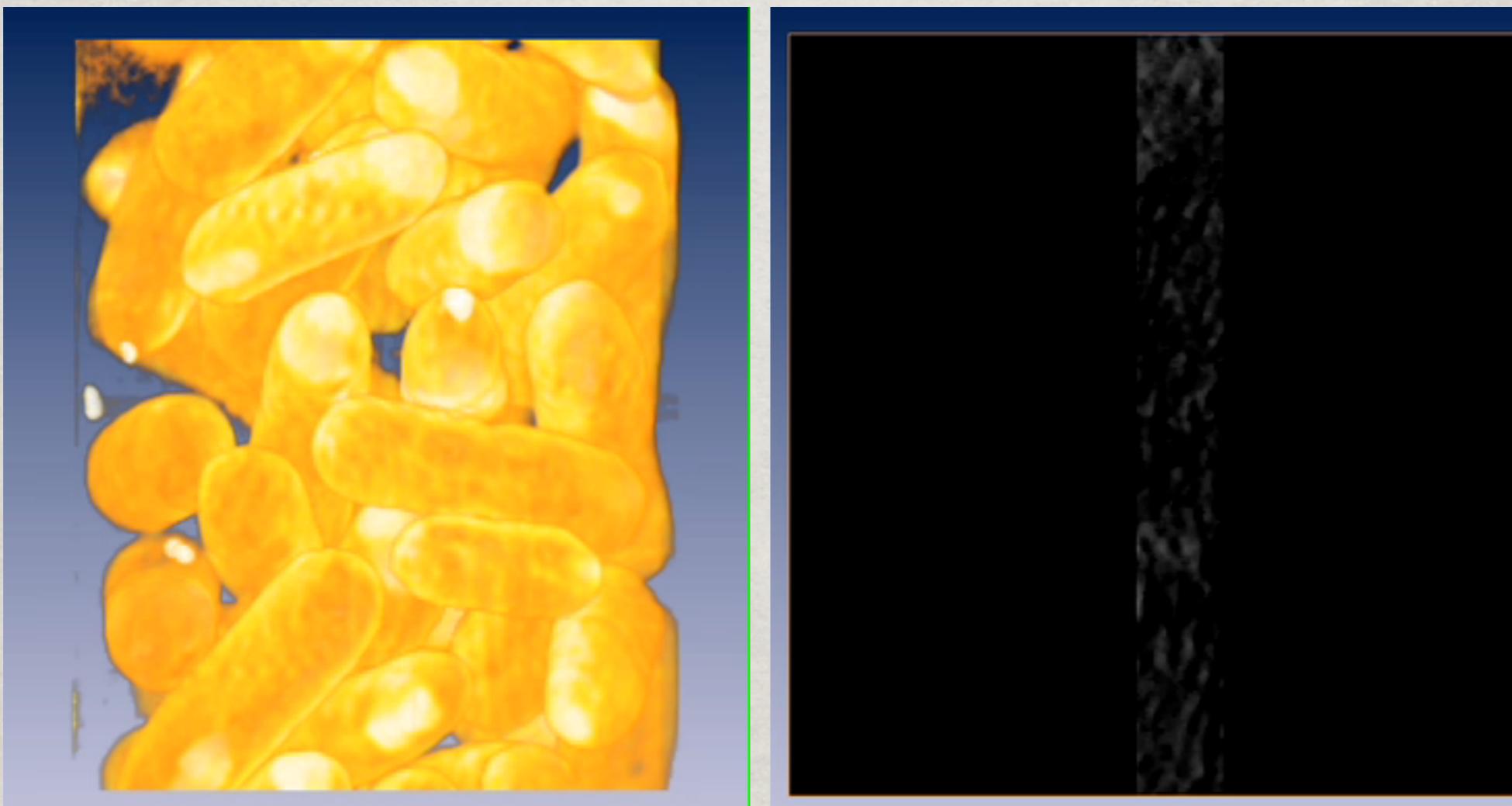


Color-coded using x-ray absorption coefficient



Color-coded using x-ray absorption coefficient

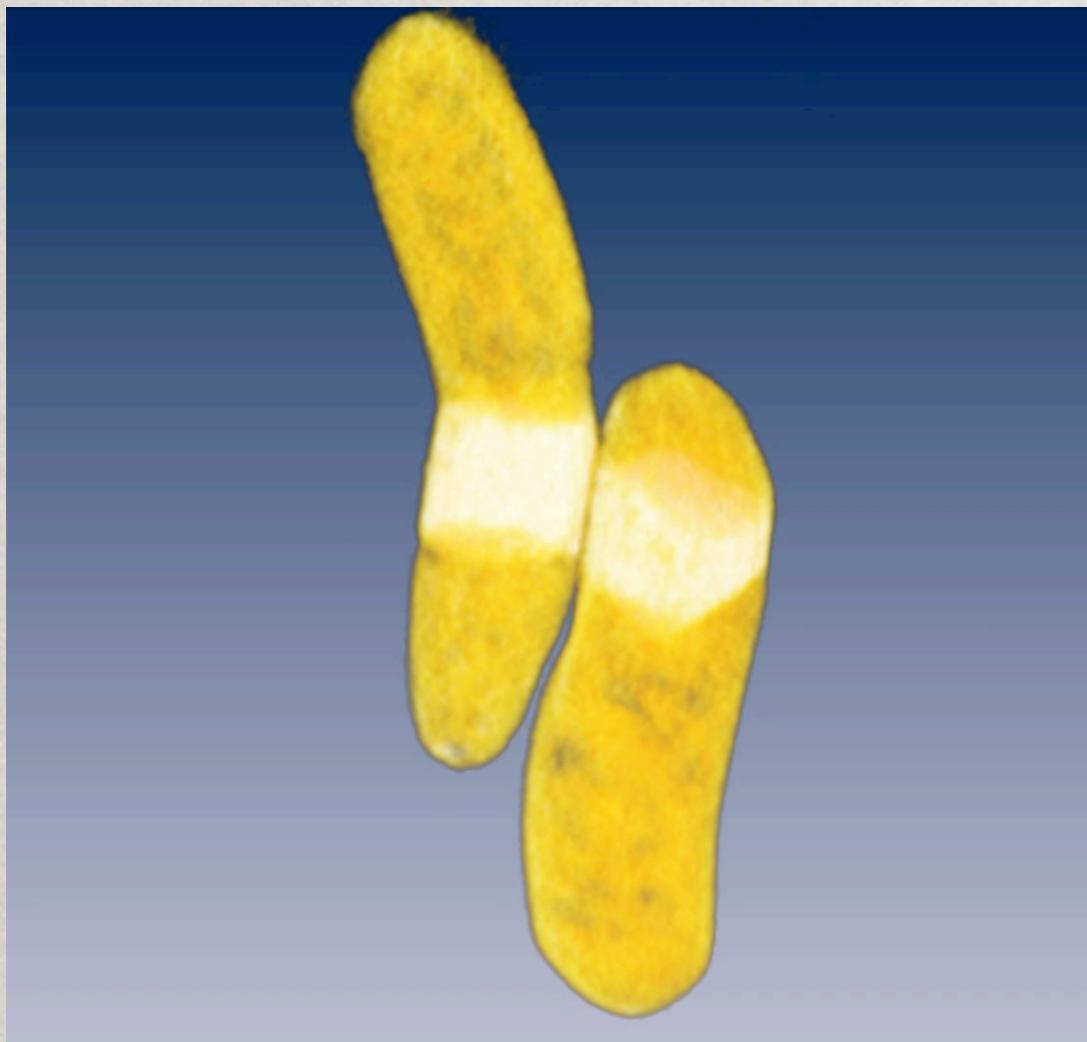
E. coli



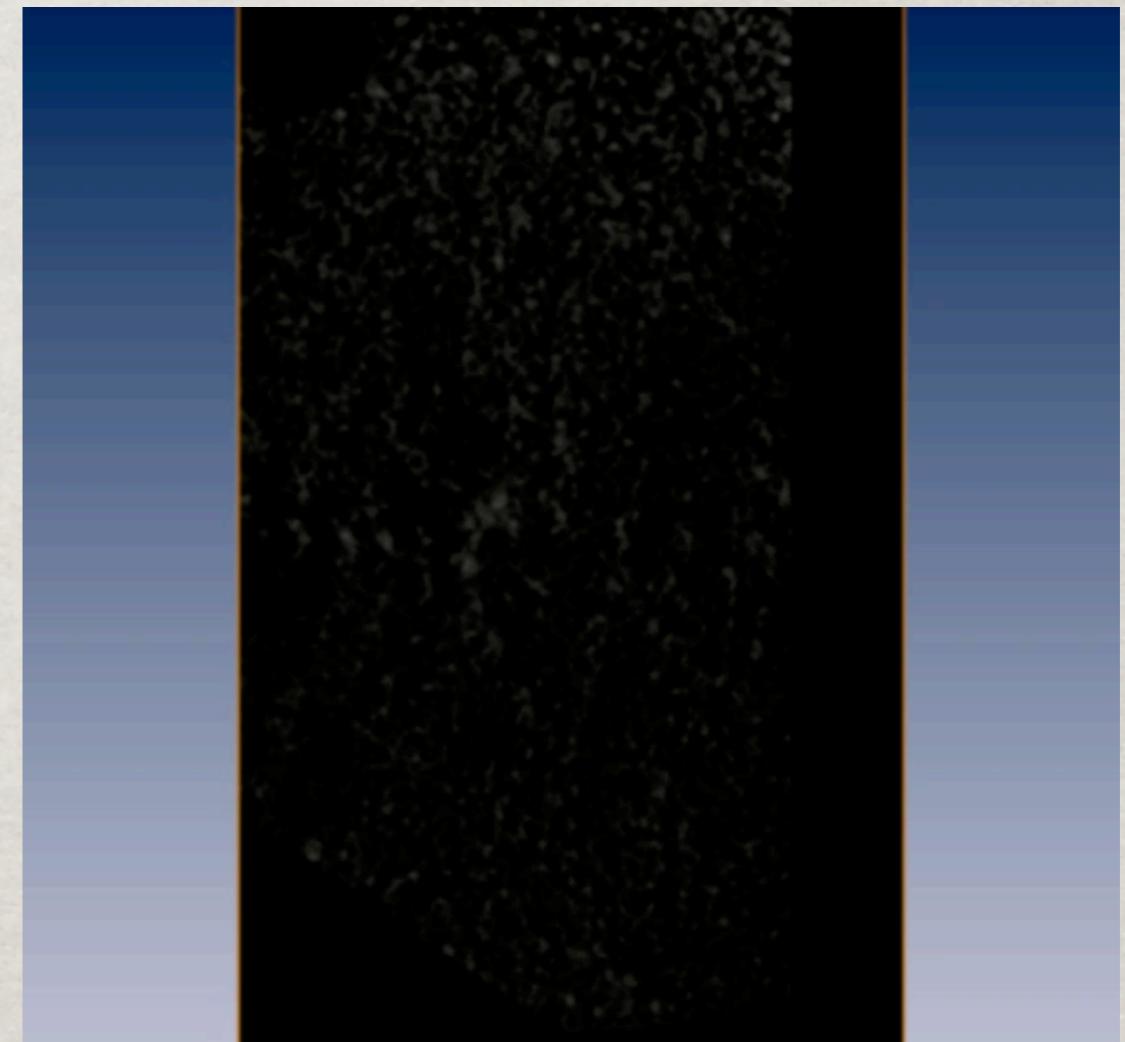
Volume rendered data set

Computer sectioning through
the reconstructed data

E. coli

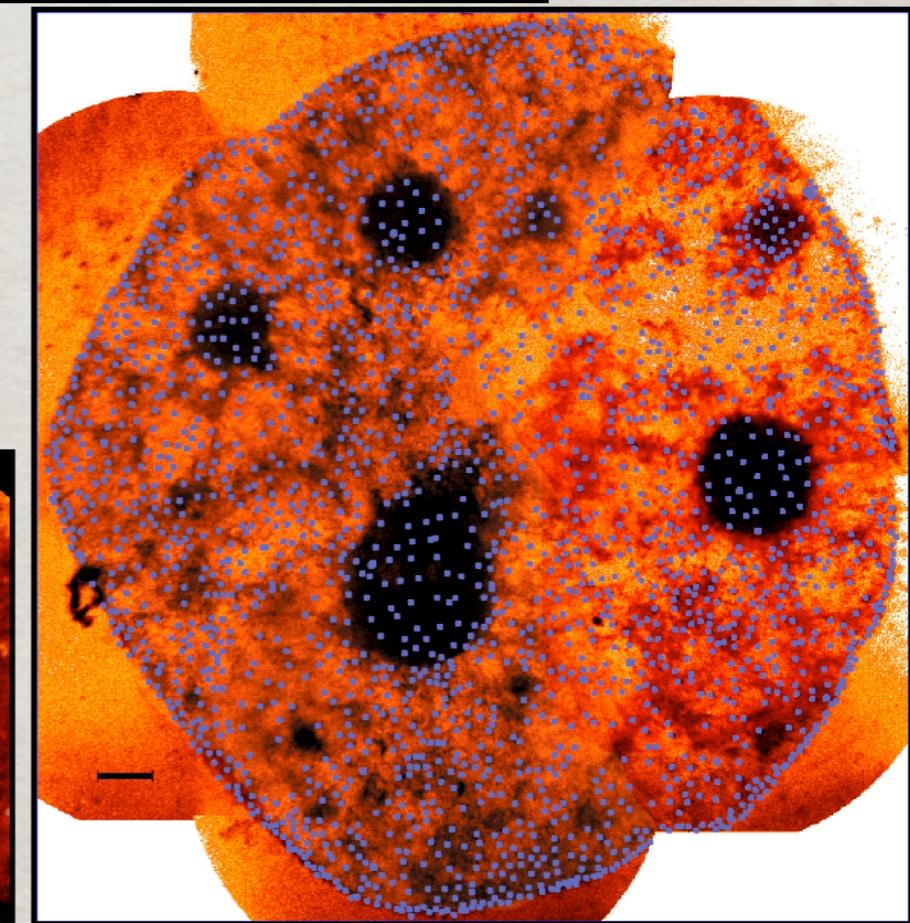
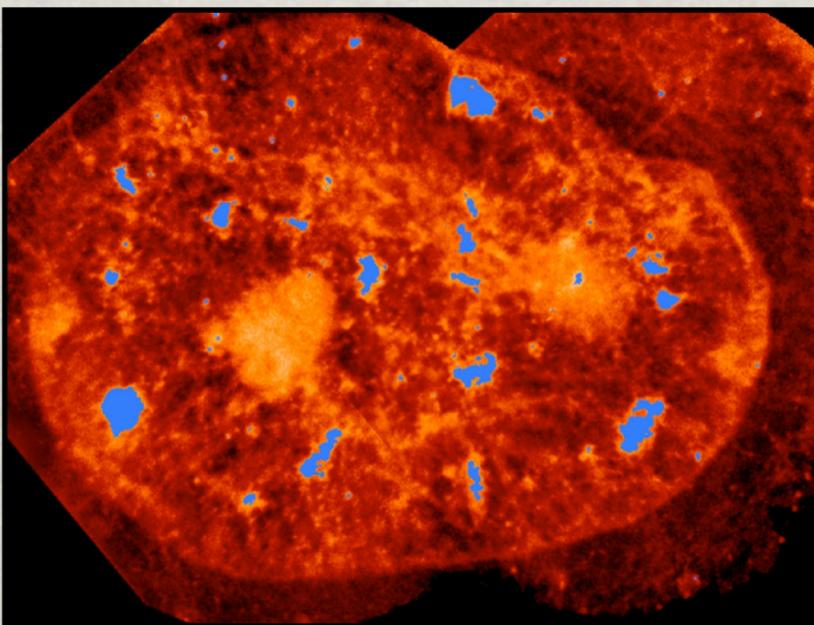
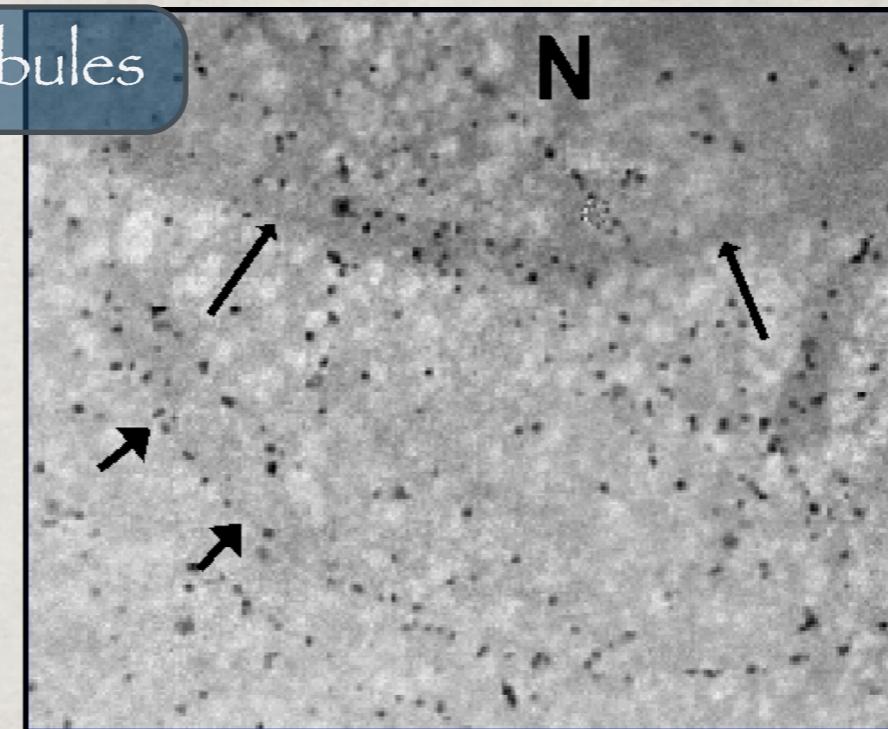
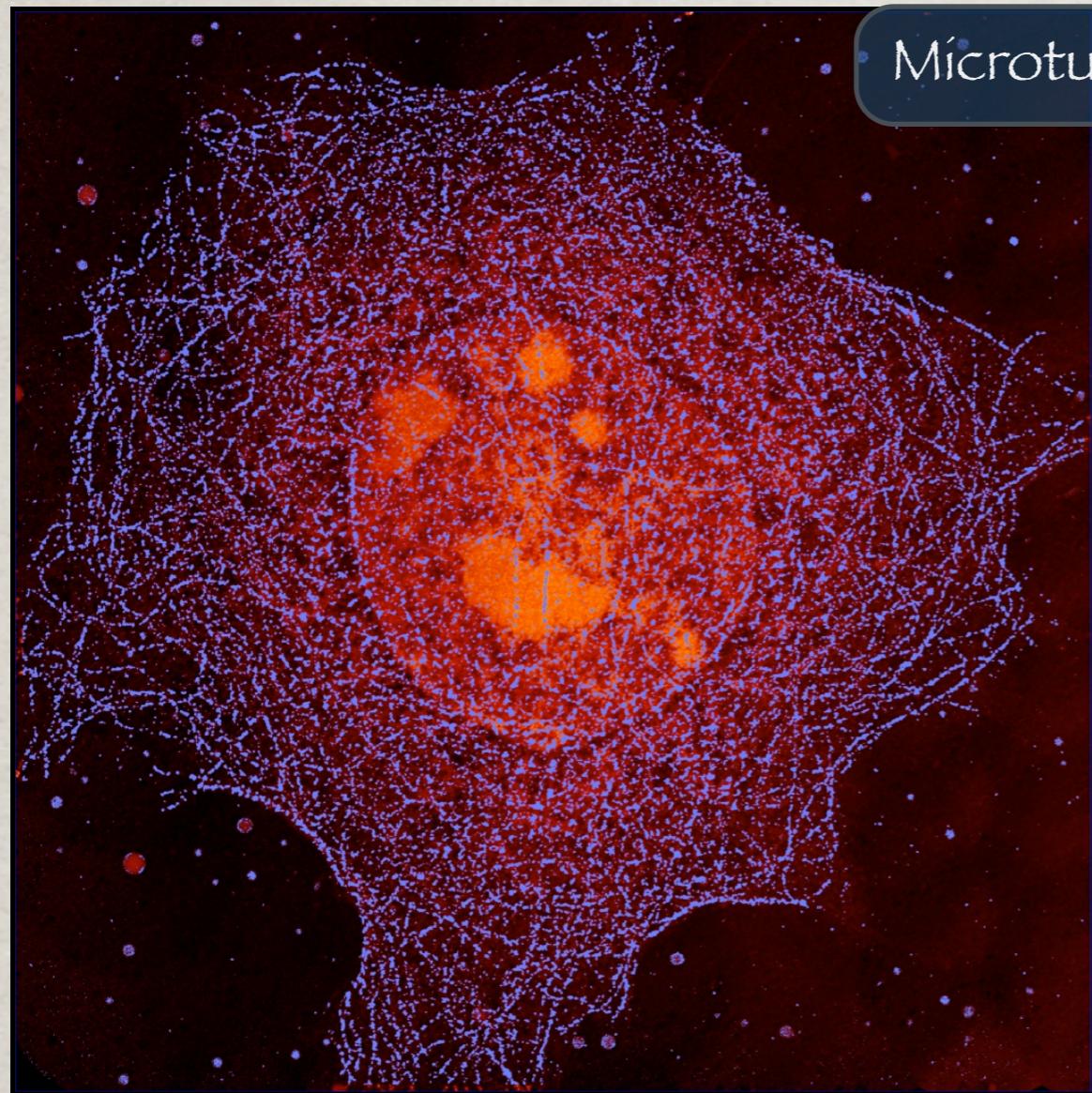


Volume rendered data set



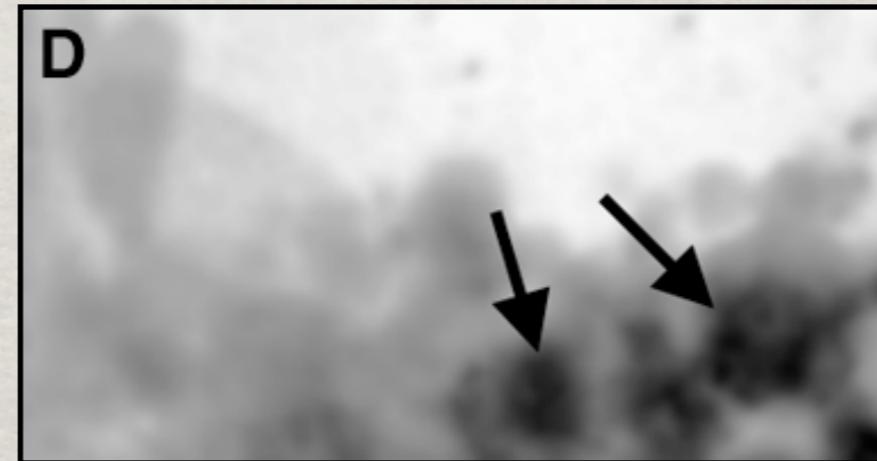
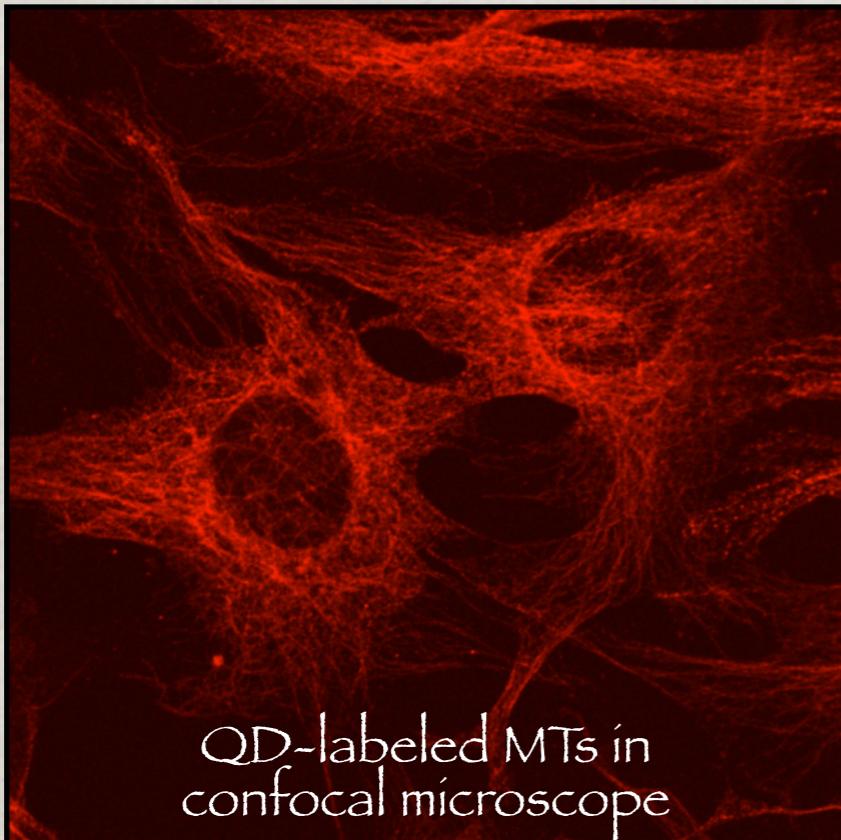
Computer sectioning through
the reconstructed data

PROTEIN LOCALIZATION: 1.4 nm gold followed by silver or gold enhancement



Localization of Molecules: New Probes for X-ray Microscopy

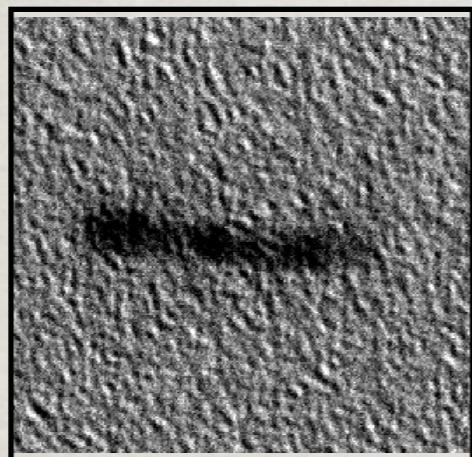
- ✿ ReAsH - biarsenical dye (Roger Tsien)
 - ✿ Track fluorescence (resorufin-based label) then examine in TEM
 - ✿ Can also use for X-ray Microscopy
 - ✿ do not need osmium - can see diaminobenzidine deposits
- ✿ Quantum Dots
 - ✿ Track fluorescence then examine in x-ray (absorbance; no processing)



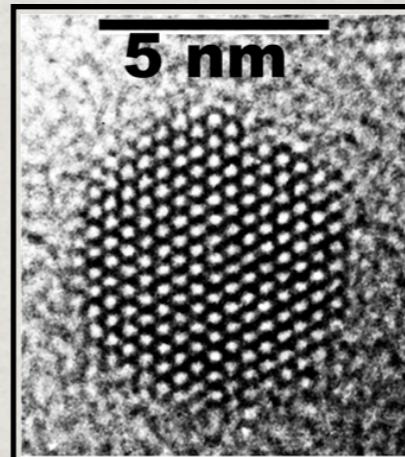
*** New metal-tagged probes (Titanium, Platinum, Terbium, etc)

TiO₂ Nanoparticles

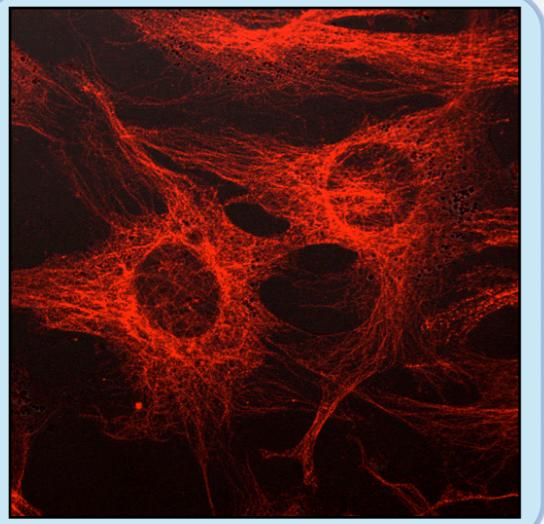
Quantum Rods



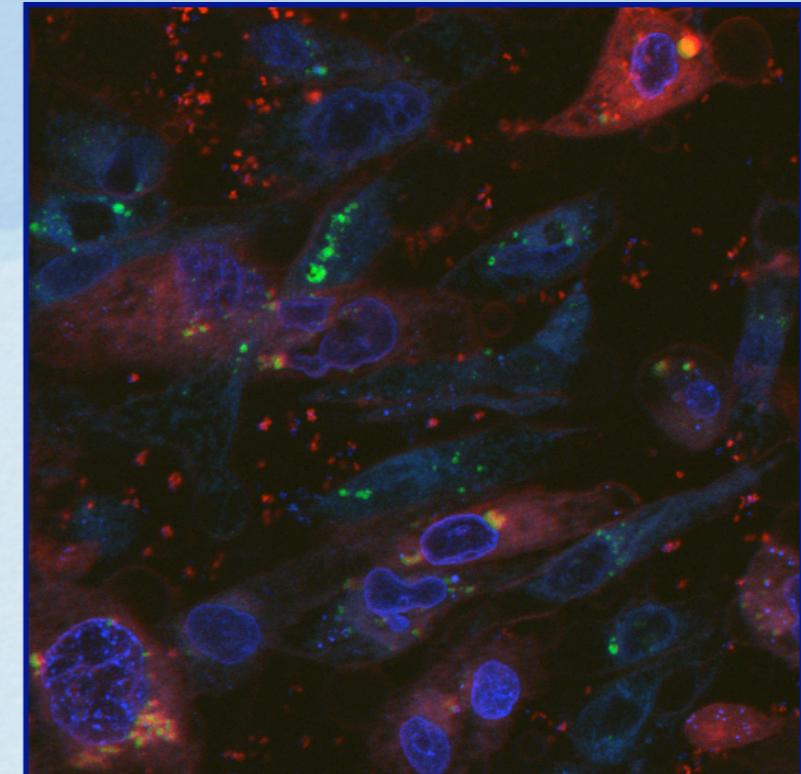
Quantum Dots



- ✿ Ti L-edge (2p → 3d, 463 eV) falls into the ‘water window’ region
- ✿ TiO₂ nano label is easily distinguishable from other labels such as nanogold
- ✿ Double labeling enables co-localization studies using Au-tagged probes combined with TiO₂ probes

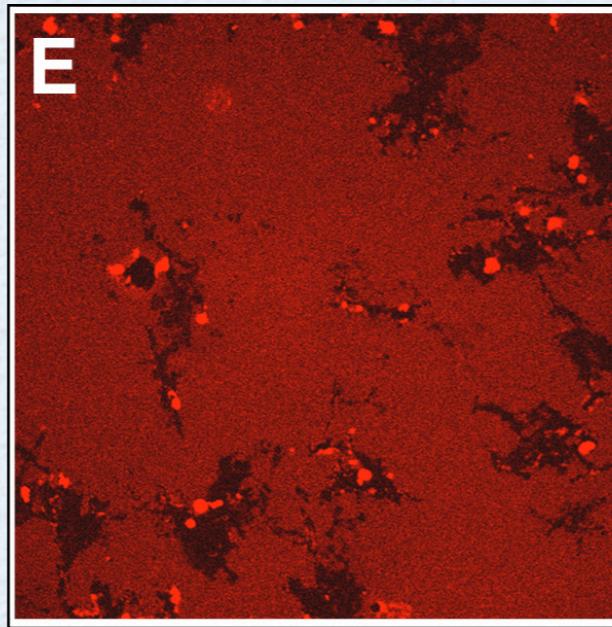
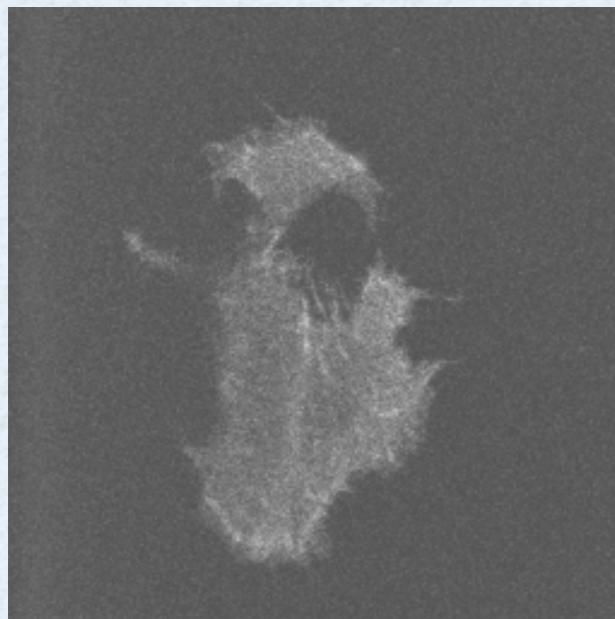


Quantum Dot-based Studies of Cancer Cell Motility, Migration, and Invasion

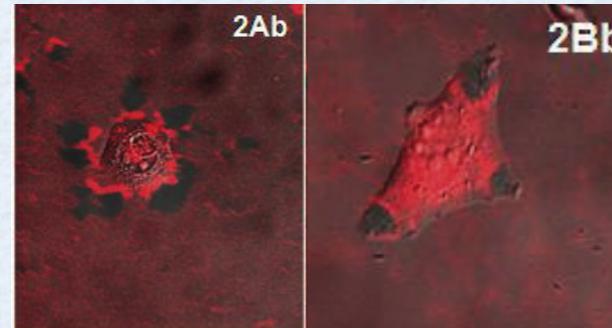
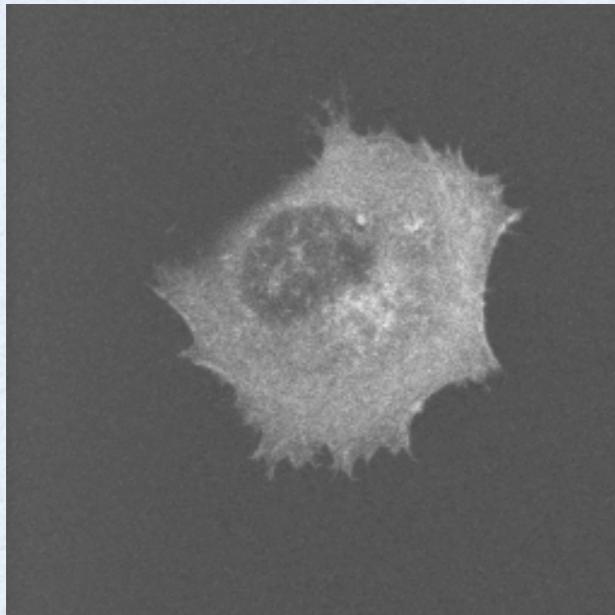


Human Mammary Epithelial Tumor Cells

MDA-MB-231 cells ("aggressive")



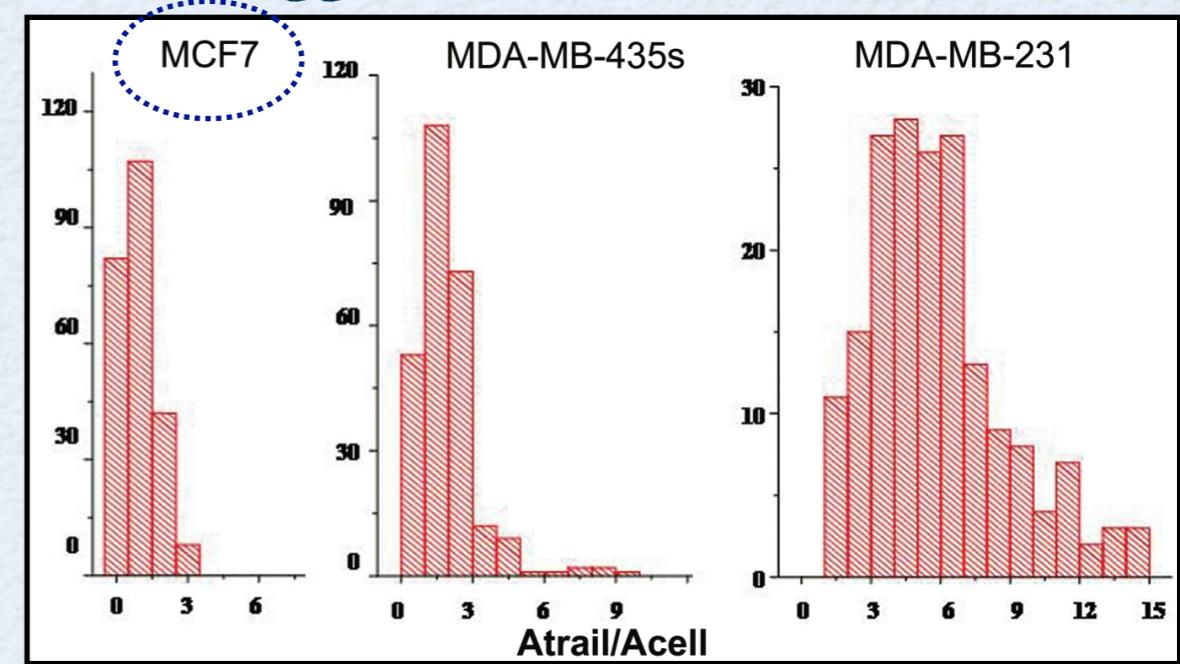
MCF-7 cells ("non-aggressive")



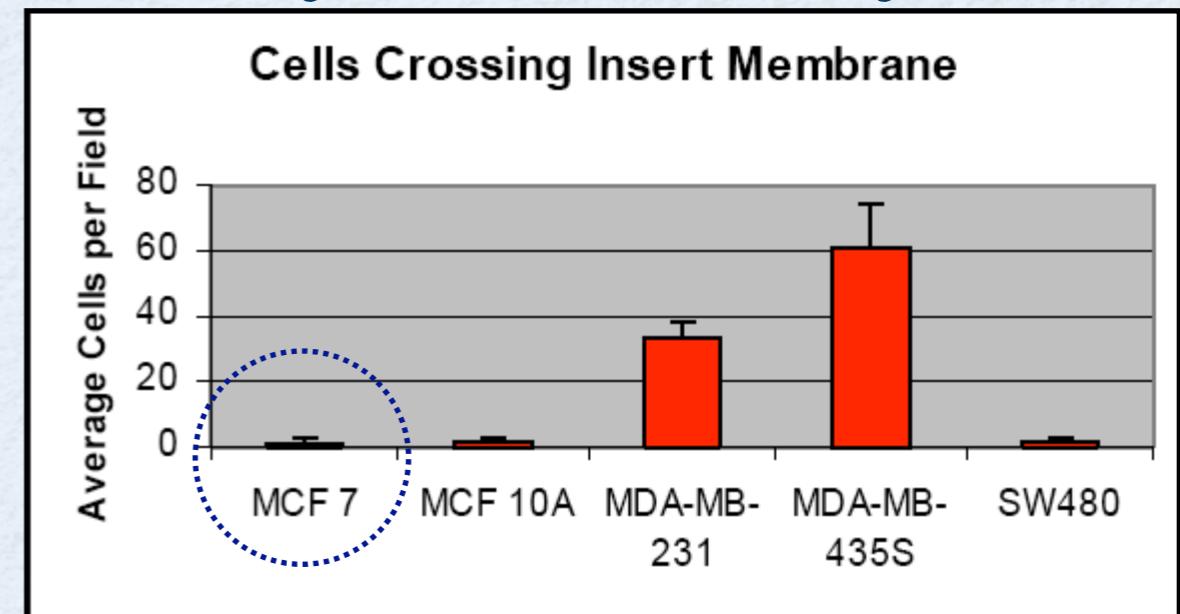
Ratio of A (trail)/A (cell)

Aggressive cancer cells: Ratio > 1

Non-aggressive cancer cells: Ratio < 1

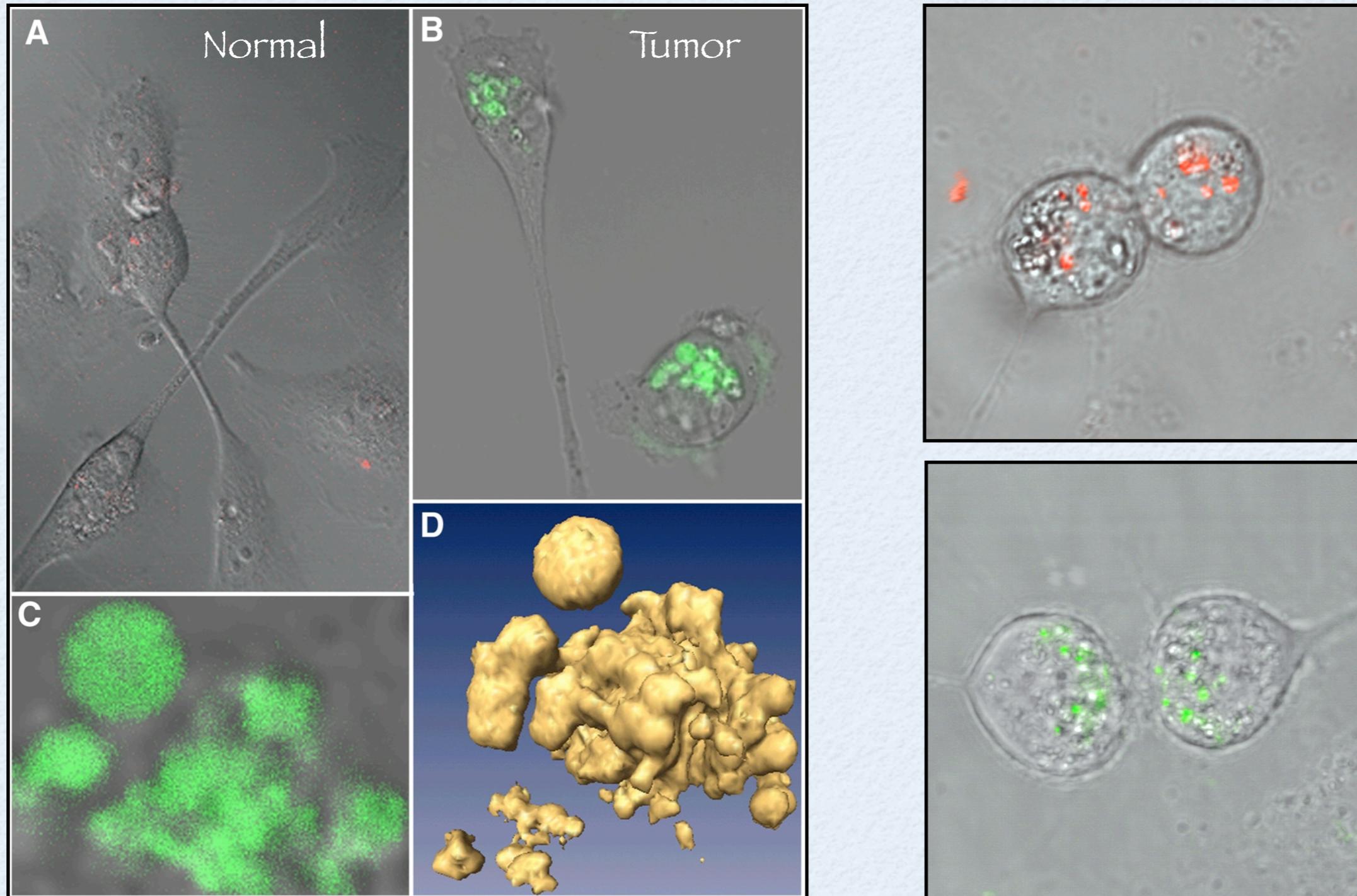


Boyden Chamber Assay



Tumor Cells engulf more QDs than normal cells

QDs passed to daughter cells at division



Imaging with X-rays: Summary

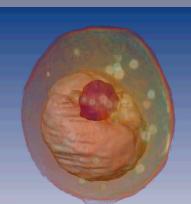
- Examine whole, hydrated cells (10 μm thick)
- Natural contrast of cellular structures (image in water window) plus labeled molecules
- Tomography: 3-D information from whole cells
- 15 - 50 nm resolution (will improve with zone plate technology)
- High throughput imaging
 - <3 min/tomographic data set
- Determine locations of proteins - gold, QDs, QRs, etc.
- Correlated light/x-ray microscopy
 - Examine dynamics of live cells in light microscope and higher resolution information in same cell with x-ray tomography

UCSF/LBNL: Mark Le Gros, Gerry McDermott, Rosanne Boudreau, Ben Engel, Weiwei Gu, Andrew McDonnell

CXRO/LBNL: David Attwood, Eric Anderson, Greg Denbeaux

UCB/LBNL: Paul Alivisatos and lab - Wolfgang Parak, Teresa Pellegrino





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National Center for Research Resources, NIH
Office of Biological and Environmental Research, DOE

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